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ON LIVER DISEASES

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PROGRAMME ON LIVER DISEASE

International Agency for Research on Cancer
(IARC), Lyon, France

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (IARC)

PROGRAMME ON LIVER DISEASE

The International Agency for Research on Cancer has had a long-standing programme on liver disease, particularly liver cancer. This programme has been concerned primarily with the various factors which have been suspected to play a role in the etiology of this cancer. There have been three main areas of interest: the mycotoxins, virus infections and alcoholism. It is probable that in the Eastern Mediterranean, alcoholism does not play a major part in liver disease.

1. The Agency's programme on the examination of the role of mycotoxins: This has been mainly concerned with field studies in Africa. The principal sites of the studies have been in Kenya and Swaziland, although the Agency has reviewed the problem of aflatoxin contamination of foodstuffs on a global basis from time to time. The results of these studies are summarized in a paper 'Field Studies on Liver Cell Cancer' **(Annex I.)** , and an additional review paper 'The Mycotoxins as a Human Health Hazard' **(Annex II.)** might prove of interest to the meeting.

Currently the Agency has a study in Swaziland in collaboration with the United Nations Environmental Programme (UNEP). This study is a continuation of earlier work by the Agency which indicated an association between **the ingestion of foodstuffs contaminated by aflatoxin and incidence of liver cancer. The principal objectives of** this study are to evaluate the association and to develop

with the agricultural services of Swaziland methods of harvesting and storage which will prevent contamination by aflatoxin. It is also envisaged that the hepatitis B prevalence in Swaziland can be surveyed and that any inter-reaction between the two factors, the mycotoxins and hepatitis B virus, can be evaluated there.

The Agency has also sponsored some studies on aflatoxin in Singapore and it appears likely that these may be augmented by national investigations in the near future.

2. Virus infections and liver cancer: The nomenclature of viral hepatitis is developing. There are now recognized hepatitis A and B with an ill-defined group: so far designated 'non A hepatitis or non B hepatitis Hepatitis A is not associated with chronic liver disease or liver cancer. The relationship of 'non A and B hepatitis' with liver cancer is still under study. However, the Agency has preserved an interest in hepatitis B virus infections ever since the demonstration of an association with liver cell cancer and with the liver diseases which of themselves are known to play a role in the causation of this cancer. Emphasis was early placed on surveys of the prevalence of the viral antigens, both in the general population and in liver disease patients. In collaboration with other units of World Health Organization, the Agency was responsible for prevalence surveys in Africa and in the Far East. At that time, it was particularly important to define the sub-types of the hepatitis virus on a global basis. I enclose a recent review of the role that hepatitis B virus may play in liver cancer, prepared by Dr Nubia Munoz of the Agency. (Annex III.)

The Agency is sponsoring a cohort study on hepatitis carriers in Singapore to assess the risk of these carriers developing liver cancer. The Chinese population of Singapore has a high risk of liver cancer and preliminary studies have also shown a high prevalence of hepatitis B infection in this population. Such studies are long-term and expensive but it is felt where reliable cancer registration exists (as is the case in Singapore) cohort studies may provide definitive evidence of the role that this virus may play in the etiology of liver cell cancer.

The Agency is conducting a collaborative study on post-mortem material from selected African, Asian, American and European countries to assess the frequency of hepatitis B surface antigen in primary liver cancer, cirrhosis and other liver diseases, using orcein staining.

Following the meeting in Kuala Lumpur in 1977 of the Regional Directors of EMRO, SEARO and WPRO, an Asian-Pacific Association for the Study of the Liver was established. This was based on the University of Singapore and was recognized by the International Association for the Study of the Liver. The International Association has for many years been a prestigious professional association of those interested in liver disease. The interest of World Health Organization and the recognition of the increasing importance of liver disease globally has encouraged the International Association to sponsor regional activities. There have been, of course, for some time regional activities as a part of the International Association in Europe and in North America, and it is now known that activities are planned for Africa under the sponsorship of Professor A.O. Williams of Nigeria.

The Asian-Pacific Association has already initiated specific programmes both for chronic hepatitis and liver cancer. A workshop sponsored by the Asian-Pacific Association on liver cancer and chronic liver disease is being held in Singapore from 20-22 October 1979 and this meeting has attracted international participation from well-known experts in this field . The Agency was not only a foundation member of the Asian-Pacific Association for the Study of the Liver, but also sponsored participation of a number of countries in the Far East at the preliminary study groups.

Experimental studies: At its Nairobi Research Centre in Kenya, the Agency carried out experimental studies on baboons to assess the role that the aflatoxins might play in liver disease. The Agency's activities at this Centre have now been concluded and the Centre has been handed over to the national authorities.

Experimental studies on the possible role of the hepatitis B virus in liver disease have also been sponsored by the Agency both at the London School of Hygiene and Tropical Medicine and the All-India Institute of Medical Sciences in New Delhi. Agency fellowships have been held in London by staff members of the All-India Institute of Medical Sciences and the collaboration between the two institutes continues.

Liver disease in Egypt: The Agency has, as you know, been interested in the prevalence of hepatitis and liver cancer in Egypt. Professor D. Trichopoulos recently visited that country as a consultant for the Agency and his final report is awaited. It had been previously reported that the overall prevalence of hepatitis was high but that liver cancer was infrequent. As one of the few situations that

challenged the association of this virus with the cancer, it will be of great interest to clarify this. The preliminary report by Professor Trichopoulos indicates that the overall high prevalence may be less than reported and that liver cancer is not as rare as suspected.

In conclusion, the Agency would be very interested in further information on liver disease in the Eastern Mediterrean region. Of the three factors that have been closely associated with this disease, alcohol would probably not be of importance in this region. The contamination of foodstuffs by aflatoxin is mainly a problem of the harvest and storage of groundnuts, maize and rice. As the most common cereal in the Eastern Mediterranean is probably wheat, this may not represent a major hazard in the region. However, it is desirable that more information is available on the status of hepatitis. This may be of particular urgency as vaccines are being developed for primary prevention. The hepatitis B virus DNA has been cloned in bacteria recently and this may offer an alternative means for the provision of material from which vaccines can be prepared. As these vaccines may become available and their use as a public health measure may have to be considered, it is essential that we have more background information on the extent of this disease in all countries.

ANNEX I.

Reprinted from *Origins of Human Cancer*

Field Studies on Liver Cell Cancer

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A sequence of events which may define the role in human disease of a dietary carcinogen from the identification of its neoplastic properties to proposals for primary prevention is suggested (Table 1). This sequence is not invariable, but, with the large number of substances being tested by a variety of laboratory techniques for carcinogenesis, it will probably indicate many problems associated with the investigation of a suspect dietary carcinogen. Many substances tested may remain laboratory curiosities as the association with man is uncertain, but if it is considered that they are available in the diet, either as additives or contaminants, accidental or naturally occurring, then field studies may be required to assess the risk.

The problems facing the transition from animal models to human studies are well recognized. The past history in man is often vague; the genetic and environmental background is most varied; there is little hope of control of diet or other variables for extended periods; observation will be short, particularly in relation to the latent period for cancer; and, above all, there are ethical limitations (Hegsted 1975). A review of the evaluation of known chemical carcinogens and their potential risks to man indicates a depressing lack of human data, and it is difficult to discover the type and standard of information needed before legislative action or other steps to prevent exposure are evoked. The evaluation of the risk of human cancer from an exposure must rely, except for rare accidents, on the measurement of the risk in populations with varying incidence. To illustrate these problems, this paper proposes to consider the studies on the risk of human exposure to the aflatoxins and their possible association with liver cell cancer.

The dramatic discovery of the aflatoxins, mycotoxins elaborated by the *Aspergillus flavus* fungi, is well known. The fatal epidemics in poultry from toxic doses were followed by reports of the potential carcinogenic properties in many animals, both accidentally and experimentally exposed. The early anxieties of laboratory scientists that this might represent a risk to man were strengthened when unequivocal liver cancer was produced in monkeys after

Table 1
Investigation of a Dietary Carcinogen

Stage	*Method*	*Agency*
1. Identification as carcinogen	tests in animals for toxicity, carcinogenicity, and mutagenicity	laboratory
2. Human exposure	dietary availability demonstrated	laboratory and field
3. Association with specific human tumor	correlation studies	laboratory and field
4. Intervention program	monitor exposure and trends of incidence primary prevention	laboratory and field
5. Eradication of the cancer	primary prevention	sociopolitical

some 6 years of exposure (Adamson et al. 1973). It was soon realized that contamination of dietary staples by these naturally occurring carcinogens was much more likely in developing countries with subsistence farming and less technically developed harvesting and storage practices. It was also from these countries that high rates of liver cancer had been reported, and in some areas the rates were on the order of hundreds of times those of Europe and North America. The global nature of the hazard of aflatoxins was evident in the reports received from many areas of the developing world, but minor changes in harvesting and storage practices could be seen to influence the danger markedly. Many of these reports were based on material from markets and communal stores and ignored the problems of bias inherent in such samplings. It was shown also that the levels of contamination of stores of dietary staples did not reflect consumption accurately. In this case as in others, housewife selection and other simple practices play an important role in protection against food hazards.

The studies which have investigated the association of the aflatoxins with liver cancer are summarized in Table 2. Most of these studies started in 1968; at that time it was felt that a preliminary assessment might be made by means of studies linking current exposure with current cancer rates. It was known that diet, storage, and cooking habits had not changed over the recent past in these rural areas of Africa and Asia, where most food is grown on small, individual farms.

Keen and Martin (1971) analyzed market samples of peanuts, known to be a good substrate for aflatoxin elaboration, and the levels of contamination were related to cancer registration in three geographic areas of Swaziland which could be expected to yield different frequencies of aflatoxin contamination. Although this study measured only one item of diet, the correlation of the frequency of contamination with the frequency of liver cancer was clear. The study by Alpert et al. (1971) in Uganda extended the range of food examined to other major dietary staples from home stores. Again an as-

Table 2
Studies Relating Liver Cancer to Aflatoxin Ingestion

Site	*Assay*	*Reference*
Swaziland	ground nut crop	Keen and Martin (1971)
Uganda	market food samples	Alpert et al. (1971)
Mozambique	food ready for ingestion	Purchase and Goncalves (1971); Van Rensburg et al. (1974)
Thailand	market food samples; food ready for ingestion	Shank et al. (1972)
Kenya	food ready for ingestion	Peers and Linsell (1973, 1976)
Swaziland	food ready for ingestion	Peers et al. (1976)

sociation between the levels of contamination of beans, maize, and sorghum, the staples most frequently involved, and a tribal distribution of liver cancer was demonstrated. The other studies in Table 2, from Mozambique, Thailand, Kenya, and a further study in Swaziland, attempted to measure the aflatoxin exposure in food ready for ingestion and to relate this to cancer incidence. The report from Mozambique, although not yet complete, allows the association to be evaluated in an area with the highest reported incidence of liver cancer. The cancer incidence in other areas is much lower, but the association between aflatoxin ingested and liver cancer rates is preserved. The study areas in Kenya, Thailand, and Swaziland could be divided into subareas. As the fungi and the elaboration of the mycotoxins are dependent on temperature and humidity, it was hoped that a differential of contamination could be established within each study area for a localized assessment of the problem. In Kenya, the homes of each subarea were randomized and samples of the total diet were collected from the nominated households, frozen, and analyzed for aflatoxin. The study continued for 21 months to cover all seasons and to assess variations in harvesting and storage conditions. Although registration of cancer was carried out for 7 years and hospital coverage was compared with that for other chronic diseases, it was known that the subarea with the lowest incidence of cancer was that with the least developed medical services. Swaziland could also be divided into altitude areas, and the subarea with the expectation of the lowest incidence was, in contrast, that with more complete medical coverage than the high-incidence subareas both in Swaziland and Kenya. A study was therefore undertaken in Swaziland, using the same food sampling techniques. The food samples were freeze-dried and flown to the IARC laboratories in Kenya. The Thailand studies were part of a country-wide survey for mycotoxins, which identified areas with different levels of aflatoxin contamination. Food was sampled from randomly chosen families in representative villages and analyzed. Significant contamination was noted in garlic, dried chili peppers, and dried fish, none of which appear in the African diet, where the most heavily contaminated foods were peanuts, maize corn, and sorghums. The major difficulty in all the studies was the registration of cancer patients, and all the authors expressed their reserve in interpreting the data. Table 3 details the registration of cancer patients in those studies which examined food ready for ingestion. The Swaziland studies relied on registra-

Table 3
Details of Cancer Registration of the Aflatoxin/Liver Cancer Studies

Risk area	*No. cases*	*Yrs. registration*	*Population*	*Crude incidence*
Kenya (low)	4	7	46,279	1.2
Thailand (low)	2	1	97,867	2.0
Swaziland (low)	11	5	100,719	2.2
Kenya (medium)	33	7	187,514	2.5
Swaziland (medium)	29	5	151,430	3.8
Kenya (high)	49	7	174,525	4.0
Swaziland (medium)	4	5	18,747	4.3
Thailand (high)	6	1	99,537	6.0
Swaziland (high)	42	5	91,471	9.2
Mozambique (high) (A)	101	1	576,782	16.1
Mozambique (high) (B)	?	3	?	25.4

tion carried out previously by Keen and Martin, who had provided a free histological service for the country and instituted an active registration program in government and mission hospitals. The first Mozambique study collected cases from the records of the hospitals in the Inhambane district of Mozambique, and the second study calculated the rate from a local hospital registration program and from records of gold miners from the study area who were working in South Africa. These levels are thought to be an underestimation, although there is evidence that the incidence in this study area, previously reported to be the highest in the world (Prates and Torres 1965), has decreased in recent years (Harington and McGlashan 1973). In the Kenya study, registration was set up in the Murang'a district using a network of hospitals and dispensaries, and record linkage was established with the major hospitals serving the area. Alpha-fetoprotein estimations were also made available to the medical services. The observations from Kenya and Swaziland are summarized in Figure 1, and the value of these data and a comparison with the other studies when updating the Kenya study using 7 years of cancer registration are discussed by Peers and Linsell (1976).

May we conclude, therefore, that the carcinogen is available and consumed in Africa and Asia and that there is at least circumstantial evidence of an association, in a number of areas, between its ingestion and liver cancer? The strength of this association will be influenced by whether the levels found in the food could be expected to produce a biological effect. The levels are low, but the potency of aflatoxin as a carcinogen must be recalled. Wogan (1975) has calculated that the highest levels from Thailand amount to 20–30% of comparable intakes which induce nearly 100% tumor incidence in rats following continuous exposure. The intakes both in Thailand and Africa are expressed as family or cluster-center averages, and individual exposures could undoubtedly be higher. The levels from Mozambique are 2 to 3 times higher than those from Thailand.

The field studies we have reviewed, although not constituting proof of a

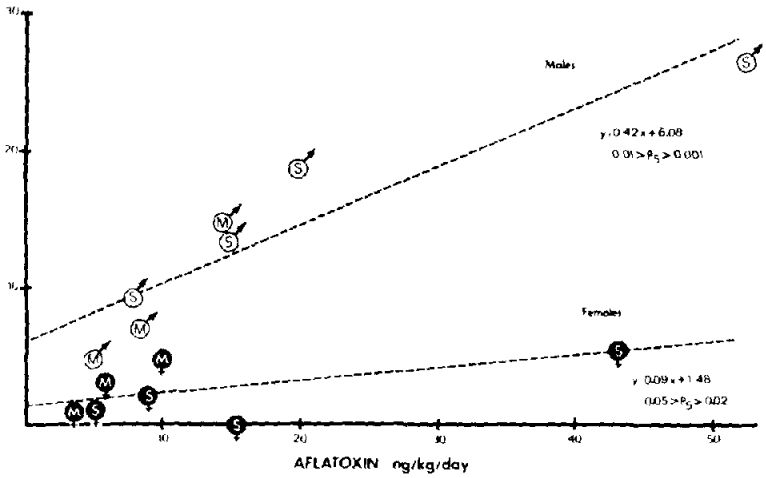

Figure 1
Combined Murang'a (M) and Swaziland (S) data. Simple linear correlation.

causal relationship, do cover that stage in the suggested sequence dealing with dose–response and justify seeking more definitive proof by an intervention program. One can consider mounting storage-improvement schemes to lower the level of aflatoxin contamination of dietary staples and monitoring the trend of cancer incidence, or one can adopt the passive role of monitoring the natural experiment which is taking place in the wake of industrialization in developing countries. The logistics of intervention, apart from the problems of the control and monitoring of aflatoxin contamination and the difficulties of registering cancer in developing countries, bring us to the often repeated litany that cancer is multifactorial and none more so than liver cancer.

The factors that have been suggested as etiologic agents for liver cancer are *numerous and as exotic as the countries in which the tumor is common.* Oettlé (1964) examined these and put his money on mycotoxin contamination and viral hepatitis, also known to be frequent in Africa. Since the demonstration of the hepatitis B antigens, the role of at least one type of hepatitis in the etiology can be studied. After the usual delays associated with the development of specific and sensitive tests for the antigens and the mapping of the global distribution of subtypes (Nishioka et al. 1975), it is now evident that hepatitis B is more common in developing countries and that the carrier state is associated with liver cancer. Perinatal transmission of the antigen is high in some communities (Table 4) (Schweitzer 1975). It has been reported from Japan that HBsAg- and e-antigen positivity in mothers is related to the development of HBsAg in the offspring. HBsAg antigenemia did not appear to develop in babies born to mothers who were HBsAg-antigen-positive but e-antigen-negative. Examination of the mothers of liver cancer patients in Senegal revealed that 70–80% of them are now and presumably were carriers of the antigen during the perinatal period of the cancer patients (Table 5) (Blumberg et al. 1975). The normal expectation of women over 40 carrying the antigen can be judged from the prevalence rates in the general

Table 4
Frequency of Transmission of HBV from HBs-carrier Mothers to Infants

Investigator	*Area*	*Pairs studied*	*Percent perinatal trans-mission*
Nishioka	Japan	250	60.0
Beasley	Taiwan	158	40.0
Schweitzer	U.S.A.	36	16.5
Papaevangelou	Greece	15	6.5
Skinhoj	Denmark	36	0
Punyagupata	Thailand	14	0

Senegalese population, where the expected level of antigenemia would be approximately 5% (Szmuness et al. 1973). The mechanisms of why the carriers are unable to deal with the antigens is unknown, and, in an acute attack of hepatitis, the persistence of the antigen does not usually exceed 3 months. We may suggest that an infection acquired perinatally could develop more readily into a chronic, persistent hepatitis, presenting a higher risk for liver cancer. A prospective study of antigen carriers is required, and any monitoring program for liver cancer must include an appraisal of the natural history of hepatitis B in the study population. Interest in the protection of other high-risk groups for hepatitis—medical attendants and inmates of renal dialysis units and other special medical services, and indeed the population at large—has stimulated much scientific effort, and the production of a protective vaccine is now considered to be a real possibility (Anon. 1976). So far, we have been dealing principally with the influence of these factors in communities, but we must now consider the problem of many being exposed and few being chosen. It has been suggested that the aflatoxin studies do not explain satisfactorily the preponderance of male victims, since, as far as we know, both sexes are exposed to the same risk from food contamination, apart from the fact that the drinking of home-brewed beer is not usual among African women. Drawing on evidence from animal experiments, there may be grounds for believing that males metabolize the ultimate carcinogen less well than females. Butler (1971) has shown that males are more susceptible than females to the acute toxicity and chronic carcinogenic effects of aflatoxin.

Table 5
Liver Cancer Patients and Their Parents

		HBsAg+	
	No.	*no.*	*%*
Cancer patients	28	22	78.6
Their fathers	27	5	18.5
Their mothers	28	20	71.6

Simultaneous diethylstilbestrol administration diminishes the carcinogenic effect in rats (Newberne and Williams 1969); hypophysectomy (Goodall and Butler 1969) and castration (Cardeilhac and Nair 1973) also inhibit aflatoxin carcinogenesis. Wherever this sexual distinction has been studied, hepatitis B has definitely been shown to affect males more frequently than females, and the carrier state itself is more frequent in males (Sherlock 1976).

It has been suggested that liver cancer is an excellent model for the study of the interaction of two well-defined factors, one chemical and the other viral. It is a tumor with an established biological marker, the alpha-fetoprotein, the estimate of which can make the definition of the individual case more accurate, particularly in those areas where biopsy and autopsy present major difficulties. The chemical agent has been stigmatized as one of the most potent hepatocarcinogens known, and more sensitive methods for its detection to allow individual exposure to be more sharply defined, or to permit more detailed studies of metabolism, are possible. The linking of the aflatoxins to larger molecules will allow more sensitive and specific radioimmunoassays to be developed (Langone and Van Vunakis 1976; Carruthers et al. 1976). There are also aspects in the suggested viral etiology which would allow individual susceptibility to be assessed. It is emphasized that although this cancer is not frequent in Europe and North America, it is in many populous areas of the world, and if the preliminary reports from the People's Republic of China are confirmed, it may be one of the world's major cancer problems. With the possibility of assessing the two factors and with prospects of active intervention, we may consider the transition to the final target of the sequence of investigation, that of primary prevention. If it is possible to implement this by reducing mycotoxin contamination and controlling hepatitis, at worst we will have eliminated two major health hazards, and at best we will have prevented a cancer, frequent in the Third World, which has at present no effective treatment.

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THE MYCOTOXINS AS A HUMAN HEALTH HAZARD

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The mycotoxins are products of fungi and are related to that important group of life-saving drugs, the antibiotics. Of those which have been thought to present a carcinogenic risk, and which are naturally occurring, cyclochlorotine and luteoskyrin are both related to the fungus Penicillium icelandicum. Although this fungus has been isolated rarely from food in Japan and Africa there is no evidence of it representing a major food hazard. Sterigmatocystin, for which there is good animal evidence of carcinogenicity, has not, however, been found as a frequent contaminant of food and there is no evidence so far of it being a human hazard. The aflatoxins, which have attracted a great deal of laboratory and field research in veterinary and human medicine, are, on the other hand, now becoming a major economic and legal issue in many countries. There are four major members of the group, aflatoxin B_1 being the most potent, and therefore that most frequently used in laboratory research. The compounds have pronounced acute toxic properties, and this was clearly illustrated by the report of an epidemic of aflatoxicosis in India in 1975 (1) where over 100 persons are said to have died.

The possibility that the aflatoxins in small doses may be related to cancer is, however, a different problem, theoretically at least always present in many areas of the world, particularly those where the people live by subsistence farming. The evidence in man of aflatoxin constituting a chronic hazard therefore indicates that a definite human exposure exists in certain areas of Africa and Asia and that correlation studies demonstrate a dose-related response when compared with cancer incidence.

To review briefly the experimental evidence in animals, it can be said that the aflatoxins are powerful carcinogens, producing tumours, mostly in the liver, in many animals, both in agriculture and in the laboratory. There is great variation in the susceptibility of animals to the aflatoxins, although as always it is difficult to correlate the varying parameters of different experiments (2) (Table 1).

The rat has been the most commonly used experimental animal and the dose response in a particularly susceptible strain is shown in Table 2 (3). At a dose of 1 part per billion (ppb) aflatoxin B_1, approximately 10% of the animals had cancer. The response is dose related to 100 ppb with cancer in all animals surviving 18 months. Tumours have been reported in rats a year after a single dose of aflatoxin and subsequent normal feeds (4).

TABLE 1. Carcinogenicity of aflatoxin

Species	Dose	Duration of observation	Tumour frequency
Duck	30 µg/kg in diet	14 months	8 in 11 - 72%
Trout	8 µg/kg in diet	1 year	27 in 65 - 40%
Tree shrew	24 - 66 mg total	3 years	9 in 12 - 75%
Marmoset	5.0 mg total	2 years	2 in 3 - 65%
Monkeys	100 - 800 mg total	over 2 years	3 in 42 - 7%
Rats	100 µg/kg in diet	54 - 88 weeks	28 in 28 - 100%
Mice	150 mg/kg in diet	80 weeks	0 in 60 - 0%

TABLE 2. Dose-response to aflatoxin B_1 in male Fisher rats

Dietary levels ppb	Duration in weeks	Liver cancer frequency
0	74 - 109	0/18
1	78 - 105	2/22 - 10%
5	65 - 93	1/22
15	69 - 96	4/22
50	71 - 97	20/25
100	54 - 88	28/28

Recognizing that a dose of 1 µg/kg will induce tumours in rats in 10% of the group exposed, the lifetime risk for rats has been computed at 240 per 100,000 at a level of 0.1 µg/kg and 1,100 per 100,000 at a dosage of 0.3 µg/kg (5). This will be relevant when we consider later the extrapolation of animal evidence to man.

From Table 1 it will be noted that the mouse is resistant to doses many times greater than those which produced tumours in rats. However, infant hybrid mice develop tumours when given repeated injections of a low dose in the perinatal period (6). This leads us to the first factors which may influence response - age of exposure as well as variation of susceptibility between species.

Several studies have shown that female rats are more resistant to both toxic and carcinogenic effects (7) and this tendency is observed even at lower doses of aflatoxin. Other factors which could influence the response in man have been examined in animal experiments. The evidence of the influence of protein malnutrition, so important in those areas of the world where liver cancer is common, is contradictory. Experiments on deficiencies of specific dietary lipotropes, such as methionine, show some protection against the toxic effects but

a higher frequency of tumours in deficient animals (8). The effects of sunlight, again, would be of interest in our consideration of the applicability of these results to man, as liver cancer is more frequent in the tropics. Rats irradiated after low doses of aflatoxin show a decrease of tumour frequency and it is suggested that endogenous photo-sensitized riboflavin may complex with the aflatoxin and inhibit the production of the ultimate carcinogenic agent (9). A protective role against the carcinogenicity of aflatoxin has been demonstrated in rats with sodium phenobarbital, and it has been suggested that induction of liver microsomal enzymes that metabolize the aflatoxin to non-carcinogenic products may be responsible (10 - 11).

To summarize the animal evidence which may relate to the aetiology of liver cancer in man, we can state that:

(a) aflatoxin is carcinogenic in many species, including monkeys;
(b) aflatoxin is a very potent carcinogen;
(c) a dose-response relationship has been demonstrated;
(d) tumours are more readily produced in males and in the young.

It was recognized at an early stage of the investigations in man that the aflatoxins, although available worldwide, would be found most commonly in hot, humid climatic conditions. It was precisely from countries with such a climate that higher frequencies of liver cancer had been reported. Investigations soon demonstrated that samples of cereals and nuts from markets and home stores in tropical countries had impressive levels of contamination. However, such high levels were not detected when food ready for ingestion was examined. Housewife selection of cereals and other simple cooking methods play an important role in protection against these hazards. Long-term studies of plate samples of food ready for ingestion were therefore undertaken, assuming that an assessment of current exposure would be relevant to current cancer rates. This is perhaps a bold assumption but it is known that diet, storage and cooking habits had not changed markedly over the years in rural Africa and Asia, where most food is grown on small individual farms. The siting of these studies was dictated by the possibility of measuring cancer frequencies, not an easy task in countries with a minimal infrastructure of health services predominantly concerned with infectious and tropical diseases. The recognition of a biological marker for hepatocellular cancer, alpha-fetoprotein, enabled a more accurate diagnosis to be made under these conditions than had previously been possible. The results of studies to assess the level of contamination and cancer frequency, both in Africa and Asia, are shown in Table 3 (12) It may be unwise to use these statistics in any sophisticated analysis without recognizing the numerous biases which might be present in such field studies. This is particularly pertinent when considering the registration of cancer cases in rural Africa and Asia. In the Kenya study the area of low frequency was that with the less well-developed medical services, and as the number of cancer cases was small, the detection of every case was vital. To check this, a study similar in design was carried out in Swaziland, where the ratio of hospitals to liver cancer frequency was reversed and where one could be more confident of case detection (14). It would appear, however, that what evidence we have in man does indicate a dose response.

TABLE 3. Summary of available data on aflatoxin ingestion levels and primary liver cancer incidence in adults

Country	Area	Aflatoxin	Liver cancer	
		Estimated average daily intake in adults: ng/kg body weight/day*	No. of cases	Incidence per 10^5 of total population /year
Kenya	High altitude	3.5	4	1.2
Thailand	Songkhla	5.0	2	2.0
Swaziland	High veld	5.1	11	2.2
Kenya	Middle altitude	5.9	33	2.5
Swaziland	Mid-veld	8.9	29	3.8
Kenya	Low altitude	10.0	49	4.0
Swaziland	Lebombo	15.4	4	4.3
Thailand	Ratburi	45.0	6	6.0
Swaziland	Low ve[illegible]	43.1	42	
Mozambique	Inhambane	222.4	460	[illegible].0**

*Excludes any aflatoxin present in native beers
**Revi[illegible] ncidence estimate taken from Van Rensburg (13)

[illegible] of the great difficulties in the transfer of the experimen- [illegible]evidence to the human situation is the disparity of levels of [illegible]xposure, and you will have noted that the human studies record exposures of nanogram amounts of aflatoxin. However, we must recognize the potency of aflatoxin, at least in some animals. It must be noted also that by the currently used physico-chemical methods, the lower level of detection is around 1 nanogram per kilogram.

It has been suggested that the exposures of man and rats may be of the same order of magnitude (15). As we have seen, a 1 µg/kg diet produces a 10% tumour frequency in rats, and if a 250 gram rat is assumed to eat 15 grams a day then the daily consumption will be 60 ng/kg. The level of daily consumption by man in the field studies in Table 3 rose in Thailand to 50 ng/kg of body weight, and at this level the tumour incidence was recorded at 6 per 100,000 per year.

It must be stressed that the observations for man are average ingestions and individual exposures could have been much higher. However, any approximation of the life risk for liver cancer in man, in this context, would indicate a much lower susceptibility and this we would expect, from some of the experimental studies which have been quoted.

There is much further research which needs to be done to test the specificity of the aflatoxin/liver cancer association. This can be

combined with studies on how the elaboration of the toxin on cereals can be prevented under rural conditions. The long-term effect of such intervention can be measured, as indeed can the risk for cohorts of those exposed to known doses of aflatoxin, but it will be many years before these natural experiments can be assessed.

There is a strong liklihood of another factor - hepatitis - playing a part in the induction of liver cancer, and another presentation at this symposium will give the detailed evidence and any possible interaction between the two factors (16).

It will be agreed that aflatoxin is a hazard - an acute one and very probably a chronic one. There is little that can be done for acute toxicity, except general supportive care and, of course, prevention.

It has been suggested that after the recognition of an environmental hazard the problems and solutions are social and economic, but this should not absolve us - the bench and field cancer scientists - from considering them. These factors may well flavour our approach to the evaluation in laboratory and field, and most certainly will affect the resources we are given to carry out our work. We must remain with the attempts to cope with the hazard, as essentially we are all interested in prevention and we can learn much from such efforts, as whilst evaluating them we may well detect other risk factors or methods which can offer a more immediate remedy. The problems associated with prevention of a naturally occurring hazard are different and more complex than those associated with a deliberate food additive or a habit or "life style" factor such as alcohol ingestion.

The global importance of the cereals which are susceptible to aflatoxin contamination include the most important of the world's basic staple foods, and there are three control situations which have to be considered:

1. Countries, like the U.S.A., with a large domestic market as well as export markets for maize and peanuts.
2. Regions, like Europe, where the main fear of this type of food contamination is from cattle feeds, with all the related problems of economic loss to those countries exporting such feeds to Europe.
3. The problem in rural communities in Africa and Asia, where liver cancer is a problem, but where the provision of sufficient food is the first priority.

In the first situation we have to work out an acceptable risk for the domestic population, and this is very difficult. The current trend is to suggest levels so low that the cost of achieving them may be great enough to affect the economics of the marketing of crops. It calls, in fact, for a difficult balance of social, economic and scientific values which perhaps everyone would like to avoid but cannot.

The situation in Europe evokes different issues. Regulatory controls can be, and have been, introduced by the importing countries, but these are difficult to implement. If it is uneconomic to satisfy the import regulations, then the income of the producers, mainly in developing countries, will fall dramatically and the ensuing loss will result in a further health hazard from lack of financial support

of medical services, as well as immediate hardship for the peanut farmers.

The third control problem, that of subsistence farming in rural communities of developing countries, may represent the greatest hazard to man from these mycotoxins. The danger of mycotoxin contamination of food is mainly related to poor storage, and little is known about the extent of disease associated with this hazard. Recognizing this, agriculturists worldwide, including the Food and Agriculture Organization of the United Nations, have initiated a number of rural programmes to study the prevention of post-harvest food losses. It is hoped that these will have a considerable impact on mycotoxin contamination in all the areas which have been considered.

The methods elaborated under these programmes will help solve the problems of bulk storage of exporting countries, as well as those of rural exposure to the mycotoxins. Better food storage, of course, will also cope with hazards from insects, rats, etc., which play an indirect role in aflatoxin contamination, but which of themselves waste enormous quantities of food.

In summary, it is suggested that the animal evidence and human exposure leave us in no doubt that, whatever the exact molecular role of aflatoxins in the induction of human liver cancer, they are a human health hazard.

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LIVER CELL CANCER AND HEPATITIS B VIRUS

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GEOGRAPHICAL DISTRIBUTION OF LIVER CELL CANCER

Liver cell cancer (LCC) is one of the most common malignant tumours in sub-Saharan Africa and in South East Asia and it is also relatively frequent in some European countries, such as Spain, Switzerland and Greece. This geographical distribution is derived from several sources.

RELATIVE FREQUENCIES

These data must be accepted with caution, since they are very rough estimates of the frequency of cancer, as they are subject to many biases, but they are the only data available for some areas of the world. The percentage of LCC in autopsy series varies from 2.4 to 6.8 % in Africa and South East Asia (1-3), and from 0.2 % to 1.5 % in Latin America, the U.S.A., Europe, India and the U.S.S.R. (4-7).

MORTALITY DATA

These data also have serious limitations, particularly because of the considerable proportion of cases diagnosed as liver cancer of unspecified origin. The proportion of these cases to the total incidence of liver cancer ranges from 1 % to 100 % in 22 countries (8). It is more than 90 % in Mauritius, Japan and Greece; it varies from 65 % to 75 % in France, Italy and Germany, and it is less than 20 % in the remainder of Europe, Israel and New Zealand (8). The combined death rate for primary liver cancer plus unspecified liver cancer coincides with previous studies based on relative frequencies and with subsequent incidence data. This combined rate is high (higher than 6.0 per 100 000 males) in Hong Kong, Japan, France, Spain, Greece and in countries in Eastern Europe; intermediate (3.0 - 5.9 per 100 000 males) in some countries in Middle and Northern Europe, and low (less than 3.0 per 100 000 males) in Northern America, Oceania and some countries in Northern Europe (8).

INCIDENCE DATA

Data from cancer registries contained in volumes I, II and III of Cancer Incidence in Five Continents (9-11) provides a more accurate, but also selective, picture of the frequency of LCC. Table 1 shows selected areas classified arbitrarily in three groups: high incidence (age-adjusted incidence rate higher than 20 per 100 000 males); intermediate incidence (rates from 5 to 20 per 100 000 males), and low incidence (less than 5 per 100 000 males). High rates are seen in populations in South and West Africa and among Chinese populations in Singapore and in the U.S.A.; intermediate rates in Malay and Indian populations of Singapore, Nigeria, Recife, Brazil, Switzerland, Spain, Poland, Maori populations of New Zealand, American Indian in New Mexico, Jamaica and Cuba; low rates are observed in other populations in the U.S.A., Latin America, Europe, white populations in South Africa, and in Israel and India.

TABLE 1. Age-adjusted* incidence rates of liver cell cancer (M: Males; F: Females)

High incidence > 20 - 100 000 males			Intermediate incidence 5 - 20 - 100 000 males			Low incidence < 5 - 100 000		
	M	F		M	F		M	F
Mozambique	133.3	38.3	Singapore, Malay	14.6	6.8	Romania, Timis	4.6	5.0
Rhodesia, Bulawayo	64.6	25.4	Singapore, Indian	11.4	6.9	U.S.A. Bay Area, Black	4.2	1.7
Singapore, Chinese	24.2	8.0	Brazil, Recife	10.7	10.3	Japan, Okayama	4.1	2.9
South Africa, Natal: African	29.4	6.9	Nigeria	10.4	3.9	Puerto Rico	3.3	2.4
Senegal, Dakar	24.3	10.0	South Africa, Natal, Indian	9.5	3.3	U.S.A. New Mexico, Spanish	3.0	2.4
U.S.A. Bay Area, Chinese	21.1	4.5	Switzerland, Geneva	9.4	1.4	U.S.A. Bay Area, White	2.8	1.4
			Poland, Warsaw City	8.5	5.8	Israel	2.5	1.4
			Spain, Zaragoza	7.8	6.2	Colombia, Cali	2.4	2.5
			New Zealand, Maori	7.7	3.6	Canada, British Columbia	2.1	1.1
			U.S.A. New Mexico American Indian	5.8	0.6	U.S.A. Connecticut	2.0	0.7
			Jamaica	5.2	1.9	India, Bombay	1.4	0.6
			Cuba	5.1	5.3	South Africa, Cape Province, White	1.2	0.6
						U.K., Birmingham	1.0	0.5

Adjusted to the world standard population
From: Cancer Incidence in Five Continents, Vols I, II and III.

TIME TRENDS

Data from the three volumes of Cancer Incidence in Five Continents have been analyzed for 37 populations in 18 countries over an average period of 8 years. In 18 of the 37 populations a statistically significant median increase of 3.7 per year for males and of 6.7 for females in 15 populations was observed. This increase was clearly identifiable in Poland, Bombay, India, Alberta (Canada), Norway, Sweden, German Democratic Republic and Finland. In the other populations a decrease was observed but it was statistically significant in only one population, Latins from El Paso (U.S.A.) (12). A decrease has also been reported among the high-risk group of mine workers from Mozambique working in South Africa (13).

SEX AND AGE DISTRIBUTION

In general, males are more prone to develop LCC than females. The sex ratio ranges from 0.9 in Romania to 9.3 in American Indians in New Mexico, U.S.A.. It is of interest that this male predominance is lost in some Latin populations, such as Recife, Brazil, Cuba, Cali (Colombia) and Spain (Table 1).

In all populations, independently of the risk, the incidence rates increase progressively with age with a tendency to level off in the older age groups. In the high incidence areas, such as Rhodesia, there is a shift towards the younger age groups. In these populations the tumour is seen not infrequently under 40 years of age, but it does not occur at this age in populations with low or intermediate rates (Fig. 1).

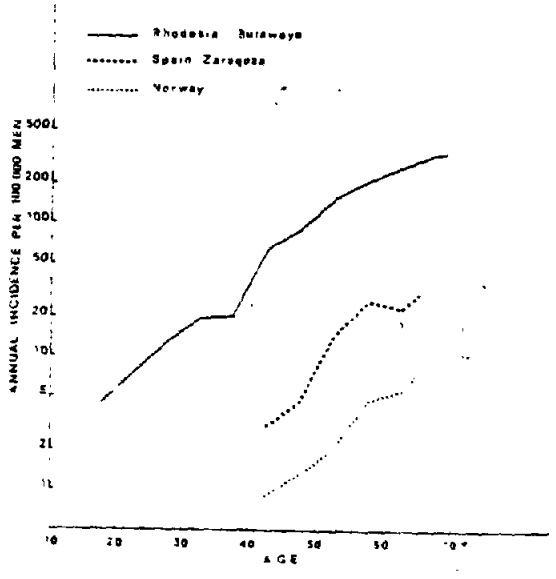

Figure 1. Age distribution of liver cell cancer

CIRRHOSIS AND LIVER CELL CANCER

An association between cirrhosis and liver cell cancer has long been recognized but it has not been clearly understood. No correlation has been observed between the mortality for cirrhosis and the mortality for LCC in different geographical areas. The highest death rates for cirrhosis are observed in Chile, Mexico, Portugal, France, Puerto Rico, Italy, Ireland and Austria, which have low rates for LCC, and lower death rates for cirrhosis are reported in Thailand, Hong Kong, Greece and Switzerland, which have high rates for LCC (WHO Mortality Data Bank). Cirrhosis is a dynamic condition of varied etiologies and with different malignant potential, which explains why no agreement has been reached concerning a morphological classification and why no correlation has been observed between death rates for cirrhosis as a whole and LCC. The simple macroscopic classification of macronodular and micronodular is useful to explain the association of cirrhosis with liver cancer. The macronodular type is more frequent in Africa and South East Asia, high incidence areas for LCC, and the micronodular type is prevalent in the low-risk areas of Europe and the U.S.A. The macronodular type appears to be more often associated with hepatitis B virus, although it is also seen in cases of reformed alcoholics, and it seems to be more prone to be complicated with LCC. It has been estimated that 40 % to 50 % of the cirrhosis in Africa and South East Asia terminates in LCC and that 60 % to 80 % of LCC arises in cirrhotic livers. On the other hand, the micronodular type is prevalent in the low-risk areas for LCC, i.e., Europe and the U.S.A., and it is often alcoholic. Only 5 % to 10 % of these cirrhoses appear to terminate in LCC, but as high as 80 % to 90 % of the LCC in these areas arise in cirrhotic livers (3-7, 14, 15).

LIVER CELL CANCER AND HEPATITIS B VIRUS

An association between hepatitis B virus (HBV) infection and LCC has been observed in several sero-epidemiological case/control studies (16-22) and they have recently been reviewed (23-24). These studies show that individuals with a chronic active infection with HBV are at higher risk to develop LCC than appropriate controls. In the earlier sero-epidemiological studies, before the identification of the core antigen and antibody, the presence of hepatitis B surface antigen (HBsAg) was used as the only marker of chronic active HBV infection. Some of these studies, in which sensitive methods (radioimmunoassay or immune adherence hemagglutination assay) have been used, are summarized in Table 2. In the study in Senegal, two groups of sex- and age-matched hospital controls were used: one non-cancer control and one other cancers control which included patients with cancers other

TABLE 2. Association between hepatitis B surface antigen (HBsAg) and liver cell cancer (LCC)

Country	No. of patients		Presence of HBsAg or anti-HBsAg		Presence of HBsAg		
	LCC	Control	LCC %	Control %	LCC %	Control %	Relative risk %
Senegal (16)	165	328	79.4	53.4	61.2	11.3	12.4
S. Africa (17)	158	200	72.9	42.4	59.5	9.0	14.9
Kenya (18)	42	450	59.5	28.7	54.8	4.7	24.7
Japan (18)	215	10738	42.3	21.2	36.7	2.7	20.6
Singapore Chinese (19)	117	150	74	78	51.0	8.0	12.1

than LCC (16). Only the non-cancer control is included in this table. The control groups for the studies in South Africa, Kenya Japan and Singapore were blood donors, or apparently healthy subjects, not matched by sex or age to the cases (17-19). It is of interest to note that the antigenaemia rates among the control groups correlate quite well with the LCC risk in these populations. Japan with the lowest rates has the lowest antigenaemia rate; Senegal, South Africa and Chinese in Singapore, with high incidence rates for LCC, also have high rates of antigenaemia and Kenya has intermediate rates both for LCC and HBsAg. The prevalence of past or present HBV infection, as evidenced by the presence of HBsAg or anti-HBs was higher among the LCC cases than among the controls in all populations and ranged from 42 % in Japan to 79 % in Senegal. However, the main difference between cases and controls is seen in the prevalence of antigenaemia with relative risks ranging from 12 in the Chinese in Singapore to 25 in Kenya.

More recent case-control studies added to HBsAg the antibodies to the core antigen as a marker of chronic active HBV infection. Studies which have used radioimmunoassay are summarized in Table 3 (20-22). The controls for the South African and Greek studies were hospital controls matched for sex and age to the LCC patients, but the controls for Zambia were healthy villagers, and for Uganda were patients with Kaposi's sarcoma or melanoma, while for the U.S.A. they were blood donors. None of these groups were matched for sex and age to the LCC cases. Again a higher prevalence of past or present HBV infection was observed among LCC patients than among controls. It ranged from 74 % in the LCC cases from the U.S.A. to 100 % among the LCC cases from Zambia, and from 5 % in the controls of the U.S.A. to 76 % among the Ugandan

TABLE 3. Association between hepatitis B virus (HBV) infection and liver cell cancer (LCC)

Country	No. of patients		Present or past HBV infection*		Active HBV infection**		
	LCC	Control	LCC %	Control %	LCC %	Control %	Relative risk %
S. Africa (20)	74	104	96.0	62.3	80.0	21.1	14.7
Greece (21)	80	160	80.0	58.7	48.8	10.0	10.4
Zambia (22)	19	40	100.0	62.5	68.4	12.5	15.2
Uganda (22)	47	50	93.6	76.0	72.3	8.0	30.1
U.S.A. (22)	27	200	74.1	5.0	40.7	1.0	68.1

* Positive for one or more HBV marker

** Positive for HBsAg (with or without anti-HBs) or for anti-HBc (without anti-HBs)

controls. A good correlation between the prevalence of active HBV infection in the control populations and the LCC risk is evident. The prevalence of active HBV infection is at most 1 % in U.S. blood donors and 0.1 to 0.3 % in the general population and LCC risk is very low. In South African blacks the prevalence of active HBV infection was 21 % and their LCC risk is very high. Striking differences in the prevalence of active HBV infection between cases and controls were also observed in all populations. Active HBV infection was defined as the presence of HBsAg (with or without other markers) or anti-HBc (without anti-HBs). The relative risks ranged from 10.4 in Greece to 68.1 in the U.S.A..

Very few risk factors associated with human cancer have given relative risks of this magnitude (heavy smoking and lung cancer RR = 10; diethylstilboestrol and adenocarcinoma of the vagina and cervix RR = 100). That this association, besides being strong is also specific, is suggested by the lack of association of HBV with other cancers (16) and with metastatic liver cancer (21). That the association does not reflect HBV infection of patients who already had LCC is suggested by the results of follow-up studies which have revealed the sequential development from acute and chronic hepatitis with persistent antigenaemia to cirrhosis and LCC (25 - 26) and by seroepidemiological studies showing that the peak of antigenaemia in most populations occurs in childhood or young adulthood (27 - 28). That active HBV infection precedes the development of LCC is also suggested by the high rate of perinatal HBV transmission from asymptomatic carrier mothers to their babies in high-risk populations for LCC in the Far East (29 - 30) and by a case-control study of 28 LCC patients and their families and 28 control patients and their families.

The antigenaemia prevalence rate in the mothers of LCC patients was 71 % compared with 14 % in the mothers of control patients (31). These data suggest that maternal transmission of HBV during the perinatal period is a crucial factor in the development of LCC.

A good complement to the seroepidemiological studies are the studies on the localization of the different antigens of HBV in fixed liver tissue. Recently methodology has been developed which makes possible the determination and localization of the different HBV antigens not only in electron microscopical and frozen sections of fresh liver tissue, but also in fixed liver tissue by immunospecific (fluorescence and peroxidase) and other empirical stains (Shikata's orcein stain) (32). It has been shown that while HBsAg can be reliably localized by immunoperoxidase and immunofluorescence techniques, Shikata's orcein stain is also specific for this antigen and offers great advantages for large scale retrospective studies (33). Since the distribution of HBsAg in the liver tissue is randomly focal, the sensitivity of these techniques greatly depends on the amount of tissue studied (33-34). Correlation studies between HBV antigens in serum and liver tissue have shown that in 50 to 85 % of HBsAg seropositive subjects the HBsAg can be demonstrated in liver tissue (35-36) and that patients with HBsAg or anti-HBc in the serum have also HBsAg or HBcAg in the liver tissue in 93 % of the cases (36).

To extend the observations derived from seroepidemiological studies a collaborative study was carried out on 679 postmortem liver specimens from patients with LCC, cirrhosis and other liver diseases from high and low incidence areas for LCC. The HBsAg in liver tissue was determined by Shikata's orcein stain. The results are summarized in Table 4. A high prevalence of HBsAg in the liver tissue of patients with LCC and non-alcoholic cirrhosis was observed in both high and low incidence areas for LCC. On the contrary, very low prevalence rates of the antigen were observed in liver tissue of patients with alcoholic cirrhosis and miscellaneous liver diseases. These findings are in agreement with previous reports from the U.S.A. (7, 36).

Table 5 summarizes the association of LCC with cirrhosis, HBV and alcohol. It can be concluded that in the high incidence areas for LCC in Africa and South East Asia, HBV is one of the main risk factors and alcohol does not play a role, but in Europe and the U.S.A. alcohol is equally, or even more important than HBV in the etiology of LCC. There are long-standing speculations that this was indeed so (5), but the identification of hepatitis B and A viruses, the association of chronic liver disease with only HBV, and the availability of markers of HBV infection, have enabled us to confirm this. For the first time the possibility of primary prevention can be

TABLE 4. Percentage of orcein positive liver diseases in high- and low-risk areas for liver cancer

Liver cancer risk	Hepatocellular carcinoma		Cirrhosis				Miscellaneous liver diseases		Total	
			Alcoholic		Non-alcoholic					
	No.	%	No.	%	No.	%	No.	%	No.	%
High-risk areas	77/99	77.8	0/4	0.0	25/44	56.8	2/44	4.5	104/191	54.5
Low-risk areas	35/70	50.0	2/130	1.5	34/120	28.3	2/161	1.2	73/481	15.2
All areas	112/169	66.3	2/134	1.5	59/164	36.0	4/205	2.0	177/672	26.3

High-risk areas: Senegal, Nigeria, Singapore, South Africa and the Philippines
Low-risk areas: Japan, India, Latin American countries, U.S.A., U.K., France and Australia

considered. In South East Asia, where perinatal transmission appears to be the determinant of LCC risk, a vaccine may not be the sole solution, and interferon therapy is being used to prevent the mother-to-infant transmission (37), but in other high risk populations, such as Greece, where the mother-to-infant transmission is not frequent (38), the vaccine may be of more immediate use.

TABLE 5. Association of liver cell cancer (LCC) with cirrhosis, HBV and alcohol

	Africa and South-East Asia	Europe and North America
Incidence of LCC	High	Low
Age group affected	Young to middle	Middle to old
Type of cirrhosis	Often macronodular	Often micronodular
Etiology of cirrhosis	Often HBV	Often alcohol
Cirrhosis terminating in liver cell cancer	40% - 50%	5% - 10%
Liver cell cancer with cirrhosis	60% - 80%	80% - 90%
Non-alcoholic cirrhosis associated with HBV	50% - 80%	30% - 70%
Alcoholic cirrhosis associated with HBV	0% - 10%	0% - 10%
LCC associated with active HBV infection	60% - 80%	40% - 50%

Aflatoxin, another recognized risk factor for LCC, has not been considered in this discussion, because it is discussed in detail in a separate paper (39). The recognized risk factors for LCC are summarized in Table 6. The first three - aflatoxin, HBV and alcohol - probably

TABLE 6. Risk factors for liver cell cancer

	Evidence of association	
	Experimental	Human
Aflatoxin	+ + +	+
Hepatitis B	-	+ +
Alcohol	±	+
Thorotrast	+ +	+ + +
Vinyl chloride	+ +	+ +
Androgenic steroids	- +	+
Oral contraceptives	+ +	+

account for most of the LCC around the world, and they may act alone or interact with each other. Exposure to thorotrast and vinyl chloride is associated mainly with angiosarcoma of the liver, but also with LCC (40-41). The nature of liver cell tumours associated with androgenic steroids and oral contraceptives is still under discussion (42-43).

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