THE AETIOLOGY OF PRIMARY CARCINOMA OF THE LIVER IN AFRICA: A CRITICAL APPRAISAL OF PREVIOUS IDEAS WITH AN OUTLINE OF THE MYCOTOXIN HYPOTHESIS*

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> 'Medical research in the tropics is still capable of yielding harvests and there are many opportunities in geographical pathology. Harvesters, not gleaners only, are still needed.'
>
> V. Ramalingaswami[1]

Since the turn of the century, primary carcinoma of the liver has been recognized as unusually common in the Cape Coloured and Bantu of South Africa.[2] Further studies have shown it to be common in many parts of Africa south of the Sahara, and a high incidence has become generally accepted as characteristic of the indigenous races of the subcontinent.

It seems logical to look for a single major carcinogenic stimulus to explain this high frequency of liver cancer in Africa. This does not deny the complex aetiology of cancer,[3] nor does it exclude the possibility of other causes such as chronic alcoholism or virus hepatitis in some cases. Nevertheless, it is better to risk the Scylla of over-simplification than the Charybdis of over-complication, if the latter becomes no more than a bewildered acknowledgement of complexity that explains away anomalies, and effectively hinders logical analysis.

Although the complete web is infinitely complex, in medicine we are concerned with effective action, and this requires no more than the identification of the relevant proximate influence which, if removed, would lead to a substantial reduction in the risk of the disease. On this empirical basis, what is diagnosed as a causal relation may turn out to be predisposing, carcinogenic (initiating) or co-carcinogenic (promoting). The factors implicated might be several degrees away from those immediately responsible in carcinogenesis, but their discovery should disclose the chain of causality whose precise links must subsequently be ascertained.

In the past a number of hypotheses have been put forward whose variety and flimsiness reflect the smattering of epidemiological evidence on which they were based. Correlation is not necessarily causation, and hypotheses, however plausible, should be subjected to crucial tests.

The recent discovery that potent hepatotoxins are produced by fungi on spoiled foods has provided yet another plausible explanation of the occurrence and distribution of liver cancer in Africa and similar areas.[4] The evidence for this is more convincing and it fits the facts more adequately than its predecessors, but nevertheless it remains no more than a working hypothesis, and much more critical examination and testing is required.

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CRITICAL EXAMINATION OF THE PROBLEM

'A theory is not a theory unless it can be disproved . . . by some possible experimental outcome.'

J. R. Platt[5]

A. Pathology of Liver Cancer in Africa

A high incidence is found to hold for one histologic type of liver tumour only, viz. hepatocellular hepatoma including those of adenoid pattern. Carcinomas of the gallbladder or of the intrahepatic or extrahepatic bile ducts are no more common in Africa than in the West. This suggests either that the agent acts specifically on the liver cell, or that it is excreted in the bile in relatively inert form, or both.

The concept of a hepatotoxic agent is supported by the common association of hepatoma with cirrhosis, although cirrhosis of the liver is not a necessary precursor of liver cancer. Where the incidence of liver cancer is high, an increased incidence of malignant change is found in non-cirrhotic livers as well. The type of cirrhosis is important. In cases with post-necrotic cirrhosis seen at postmortem in Johannesburg, malignant degeneration had occurred in over 50%, whereas it was much less common in septal cirrhosis.[6]

The proportion of cirrhotics undergoing malignant change is also higher in areas of high incidence. Thus, in Lourenco Marques the figure is 61·5% in males and 51·4% in females,[7] whereas in Johannesburg the figures are 47 and 12% respectively.[8]

Both in Lourenco Marques and in Johannesburg the proportion of elderly cirrhotics undergoing malignant degeneration is less than that in the younger group. It is possible that this is an artifact; e.g. it may be that elderly patients with a relatively acute disease such as liver cancer may be less likely to present themselves for treatment by comparison with those with a more prolonged illness. On the other hand, this trend may reflect a lessened intensity of exposure in the older group, or a greater proportion of other forms of cirrhosis, with lowered risk of malignant change. In any case, this merits investigation. It is noteworthy that in American Negroes, Miyai and Reubner found hepatoma to be a commoner complication of cirrhosis (13·4%) than among Whites (7·4%). This they attributed to the relatively high incidence of post-necrotic cirrhosis among Negroes, rather than to any racial factor.[9]

The situation in Uganda is unusual. Here Steiner and Davies[10] have reported that 21% of carcinomas arose in livers with no cirrhosis at all, and of those associated with cirrhosis the association was chiefly with Laennec cirrhosis (73%) rather than with acute post-necrotic scarring (22%). Evidence of regenerative hyperplasia was less in the Uganda livers than in the American, and they concluded that the liver cancers did not arise in the hyperplastic nodules.[10] Such facts, combined with comparatively low rates of liver cancer, suggest that the picture here may be exceptional.

In the West, primary carcinoma of the liver following upon cirrhosis tends to develop at the end of a long illness, and may be discovered unexpectedly at postmortem as a complication of the underlying chronic disease. In Africa, however, many cases are of acute onset in people who previously appeared to be completely healthy, and, in the case of gold-miners, may have successfully passed 3 separate medical examinations a few months previously. In some the first evidence of an abnormality is the terminal intraperitoneal haemorrhage. Others, on the other hand, run a chronic course similar to that familiar in the West, and all intermediate gradations are known.

Its occurrence in young persons, the rapid clinical course, as well as the annual and seasonal fluctuations, all suggest a remarkable rapidity of carcinogenesis—not so rapid, however, as to obliterate the differences between Bantu miners of different origin, for these persist while they are in employment on contracts of approximately 9 months on the mines on identical diets and conditions of life. Nevertheless, in primary carcinoma of the liver, carcinogenesis seems to be shorter than in other types of carcinoma, for it has been noted that Japanese immigrants to Hawaii do not carry with them any of the high risk of liver cancer which the Japanese population in the homeland displays.[11]

The association of hepatocellular carcinoma in Africa with the hyperplasia of post-necrotic cirrhosis, suggests that the cancers follow exposure to an agent producing necrosis followed by reactive hyperplasia. This is analogous with the phenomenon of promotion in epidermal carcinogenesis. Maltoni and Prodi[12] have shown that cirrhogenic treatment of rats with carbon tetrachloride and butter yellow simultaneously, produced an earlier appearance of hepatic neoplasms than in controls treated with butter yellow alone. Furthermore, after a subliminal dose of butter yellow, subsequent treatment with carbon tetrachloride induced the appearance of tumours. In animals previously treated with carbon tetrachloride and subsequently given butter yellow, the onset was delayed by comparison with those treated with butter yellow alone. This study indicates that the two-stage theory of carcinogenesis is applicable to the rat liver, and if it holds for man as well then it may be necessary to postulate also a prior exposure to initiating agents. This mechanism is consistent with the acute onset already mentioned. The occurrence of cases without cirrhosis demonstrates that this agent may have a direct influence without producing scarring of the liver.

Predisposing factors may also be relevant. A low level of serum albumin is commonly found in the Bantu, the albumin-globulin ratio being reversed in 69% of adult Bantu males in Johannesburg,[13] which may account for a greater susceptibility to hepatotoxic influences. In addition, abnormalities in liver-function tests were common, being detectable within the first 2 years of life.[14] This remains pure speculation, however, and we have no evidence that the risk of liver cancer is greater in persons showing these abnormalities than in those who do not.

B. Aetiologic Factors

1. *Ethnic Group*

This disease was once regarded as a feature of pigmented races, but, as with so many other examples of racial predisposition, it is now accepted that this association with race is an expression of differential environmental exposure.

The high incidence affects the negriform race chiefly, and to a lesser extent the mixed group. It does not involve Whites or Asians living alongside them, nor their domestic animals. Within the Bantu and West African Negro groups the risk is not uniform. Among South African Bantu gold-miners, for example, those from Portuguese East Africa have an incidence

which is 6 times that of the South African recruits.[15] In some regions this cancer accounts for more than one-third of all cancers recorded in both sexes, e.g. Mocambique, Congo Republic (Brazzaville), Senegal, Gambia, Portuguese Guinea, Mali, Niger and Togoland. The disease is relatively rare in countries of North Africa, viz. Egypt, Tunisia and Morocco.

The incidence of primary cancer of the liver in the Johannesburg Bantu 1953-55 survey in males was 13.5 compared with 3.4 and 3.6 per 100,000 *per annum* in United States Whites and non-Whites respectively.[16] In females it was 5.4 compared with 2.0 and 1.7. The male rate was thus 4.0 times and the female rate 2.7 times that of United States Whites of the same sex.

The situation in Uganda is particularly interesting: 5.9% of cancers are recorded as originating in the liver, which is one of the lowest figures recorded in an African series.[17]

A further interesting comment is made by Davies, who found that the incidence varies with tribe, the rate for the Ganda being the lowest, viz. 1.0% of postmortems, while that for the remainder is 2.5%.[18]

The rates reported in the Kyodondo survey are considerably less than those in Johannesburg, being 6.2 per 100,000 in males and 2.2 in females (adjusted to the African standard population).[19] Examination of the basic data, made possible by courtesy of Professor Davies, shows that the curve in Kyodondo males flattens out after 45 years whereas the Johannesburg rates show a steady rise with age. This might have suggested under-registration of older cases, but can in fact be explained on the superimposition of two separate populations, i.e. the local Baganda with a low liver-cancer risk, and a migrant group of young adult males who have a much higher risk. The rate for all liver cancers (WHO 155 and 156 excluding bile ducts and gallbladder cancers) in the Baganda is 4.9 per 100,000 in males and 2.5 in females, and tends to rise with age. The rate in the non-Baganda males is 10.9.

Comparable incidence figures are not yet available for the Coloured population in South Africa, although the scanty figures from the Johannesburg rate survey in 1953-55 indicated a high incidence, viz. 6.4 and 2.5 times the United States rates in males and females respectively. The evidence from the Cape Town postmortem series suggests that the disease is much less common among Coloureds there, occurring in 0.3% of autopsies on Coloureds over the age of 10 years as compared with 5.3% of autopsies on Africans.[20] Nation-wide mortality figures for Coloureds in 1949-1958 (standardized to the US population of 1950) showed the Coloured risk 2.9 times and 1.5 times the US White male and female risks of cancer of the biliary passages and liver. (The latter figures are somewhat diluted with cancers of the biliary passages, and are also subject to diagnostic errors.[4])

Such variation within races in Africa does not support a genetic explanation.

2. *Region*

Extraordinary fluctuations in incidence are noted between relatively adjacent areas where tribal differences are not significant, e.g. liver cancer is common in the southern Lowveld, and rarer in the northern Lowveld and the adjoining mist-belt of the Middleveld.[21]

3. *Sex*

Males are affected 4 - 5 times as often as females, in regions of high as well as of low incidence. An exceptionally high male-to-female ratio was reported from Madagascar, where Brygoo[22] recorded 24 males to only 1 female case, but this would need to be confirmed in a larger series.

This suggests either greater susceptibility or greater exposure of males.

A greater susceptibility of males to hepatocellular hepatoma has often been noted experimentally in rats where protein-deficient males are more susceptible to hepatoxic alkaloids than females,[23] and also to azo dyes, e.g. p-dimethylaminobenzene-1-azo-1-naphthalene and p-dimethylaminoazobenzene.[24] With p-dimethylaminobenzene-1-azo-2-naphthalene the incidence did not differ significantly between the sexes, but the tumours were much slower in appearing in females than in males.

More male Mastomys were affected by severe bilharziasis than females.[25] It has been assumed that in general males of all species are more susceptible to hepatoxic factors, but this deserves much closer examination.

In C3H mice, Andervont[26] has shown that males are more susceptible to the occurrence of spontaneous hepatomas (49% as compared with 3%), but females are more susceptible to the hepatomas induced with azo dye (70% in females as compared with 57% in males) and both sexes are susceptible to hepatomas induced with carbon tetrachloride (79% of males and 29% of females).

It seems wiser to withhold judgement on whether a general male susceptibility to liver cancer exists. In cancers of other sites a higher male-to-female ratio has tended to reflect greater male environmental exposure. For many dietary carcinogens, males would tend to consume more because of their greater physical demands, and differences in food habits might also expose males to greater concentrations of carcinogen.

4. *Age*

Initially liver cancer in Africa was thought to be a disease of young men, but that was because mining was an occupation of young men. In general the incidence rises with age. This is evident in Johannesburg,[27] but in Lourenco Marques the curve tends to flatten or decline slightly after the age of 45 years.[7] This might be explained on failure to register the older age-groups adequately.

In the regions of high incidence, cases occur in the first decade, but in regions of intermediate intensity the youngest cases tend to occur after puberty.

The youthfulness of the cases seen on the Rand and in the Far East led Des Ligneris[28] to conclude that 'It seems very likely that liver cancer develops on the basis of a factor which has affected the patient in his childhood days', since knowledge of industrial cancers indicated 'that usually a considerable period elapses between the time of the original irritation . . . and the actual formation of a cancer'. Children are certainly exposed to the carcinogen, and the increasing risk with age suggests lifelong risk of exposure to the carcinogens.

5. *Social Class*

Apart from ethnic grouping, no obvious correlation with social class has been demonstrated. The condition seems to be indirectly linked with poverty.

Indians and Coloureds of similar social class level differ widely in risk.

6. *Secular Fluctuation*

In 1953 and 1954, in Johannesburg, a seasonal fluctuation was observed in registrations with liver cancer, more admissions being noted during December - May than during June - November.[29] This was also noted in 1959.

In 1948 an extraordinary drop in primary cancer of the liver was apparent in figures from French West Africa.[30] This affected both sexes, the proportion of all cancers assigned to the liver falling from a level of 30% in 1947 to 9% in 1948, returning to 34% in 1949.

These fluctuations, if real, suggest that the carcinogenic stimulus is relatively inconstant in intensity, and also implies a relatively short latent period.

7. *Hormones*

Davies[18] has suggested that hyperoestrogenation was prominent in Africans, on the basis of the apparent high incidence of cancer of the male breast in Africans (a claim which is open to question[27]), and the fact that oestrogens are capable of inducing hepatomas in animals.

There is evidence that the oestrogen metabolism of the Bantu differs from that of the Whites. The Bantu have a higher oestradiol excretion while 17-oxosteroids are uniformly decreased and reach a very low level in primary carcinomas of the liver. The total oestrogen excretion in liver cancer patients, however, did not differ significantly from that of controls. Bloomberg *et al.*[31] concluded that the Bantu may be exposed to greater amounts of circulating biologically active oestrogens, but what bearing this has on liver cancer, if any, is not obvious, and no striking association of liver cancer with

gynaecomastia has been recorded. The lower rate in females would also appear somewhat anomalous if oestrogens were especially significant.

8. *Alcohol*

In White populations, chronic alcoholism is an important factor in the development of cirrhosis and subsequent malignant change. It can be excluded in the African cases for the following reasons.

(*a*) Fatty change is strikingly absent in the Bantu cirrhoses associated with hepatoma.

(*b*) Questioning of Bantu cases of liver cancer in Johannesburg showed no retrospective association with alcohol.

(*c*) No association with haemosiderosis has been found. (As a result of brewing in iron receptacles, the South African Bantu drinker has a high iron intake, since the acid fermentation may dissolve up to 85 mg. of iron/100 ml.[32])

(*d*) The occurrence of childhood cases would seem very strong evidence that the concoctions, and other alcoholic drinks other than fermented porridge, can be excluded as possible aetiologic agents in the Bantu. This was accepted by Prates, who initially had held that the drinking of alcoholic concoctions was responsible for liver cancer.[33] He concluded that the occurrence of cases of cirrhosis and primary cancer of the liver in young children 'would seem to contradict the idea that such tumours are solely due to those concoctions', although he still considered concoctions worthy of study, together with schistosomiasis and native drugs.[34]

9. *Siderosis*

Despite the known association of liver cancer with haemochromatosis, where 18% develop primary liver carcinoma,[35] no association has been noted with haemosiderosis of the Bantu, in which condition regenerative nodules are rare.

10. *Malnutrition*

The view that malnutrition is a factor in cancer of the liver seems to have been based on the high incidence in certain underprivileged races, and on the experimental evidence for the importance of certain specific nutritional deficiencies in the development of hepatomas in rats.

Many workers have favoured this hypothesis, Kraybill[36] concluding from the inhibition of liver tumorigenesis experimentally with riboflavin-rich diets, that 'This preponderance of evidence on hepatomas associated with low vitamin-B diets can probably account for the high tumour incidence among the Bantu and certain Asiatic groups'.

Malnutrition is a vague concept, and human liver cancer has not been clearly associated with any particular form of malnutrition, nor with any particular form of diet. It has been suggested that malnutrition in infancy renders the liver more susceptible to later hepatic stresses, but a high incidence of kwashiorkor is not necessarily followed by a high risk of liver cancer, e.g. in Egypt, Greece and South America. There is no evidence of quantitative differences in malnutrition between areas of high and of intermediate liver cancer incidence. The association with malnutrition may be coincidental or cognate, but the suggestion that it is of causal significance lacks valid evidence.

11. *Dietary Toxins*

Dietary carcinogens might well explain racial differences. One would tend to look for such carcinogens in the carbohydrate fraction, as this forms a much greater proportion of the diet of the poorer groups. The agent should be commoner in inferior brands of carbohydrate, but no association with any particular carbohydrate source is permissible, as the staple diet varies with region. Alternatively the agent might reside in some common additive such as spinach, or an occasional additive like a fruit or nut.

The most promising of these are the toxins produced by moulds, which are discussed later, but many other hepatotoxic agents are known, to which underdeveloped groups are exposed.

(a) *Inorganic chemicals.* Potassium dichromate is occasionally used by the Bantu in South Africa as an emetic or purgative, as well as a witchdoctor's medicine.[37] At postmortem examination on one case (case 11): 'Sections of the liver showed the presence of focal necrosis with many bile thrombi and bile casts; the necrosis was massive in places'. In another patient (case 4), jaundice persisted for 3 months and the patient was 'almost certainly developing cirrhosis of the liver when he was discharged'. There are no grounds for regarding this poison as significant in the causation of liver cancer.

(b) *Bacterial toxins.* Since the previous century, severe, often fatal poisoning has been recognized in the province of Banjumas on the south coast of Java following the consumption of a fermented coconut product termed 'bongkrek' or 'semaji'. Mertens and Van Veen[38-40] traced this to a highly unsaturated fatty-acid-like substance, bongkrek acid, produced by the action of a Gram-negative flagellate bacillus. This leads to hypoglycaemia associated with failure of glycogen synthesis in the liver, closely resembling that of synthalin (decamethylene diguanidine) intoxication.[41] There is no reason at present to suspect a bacterial toxin of this type in African liver cancer.

(c) *Plant toxins.* A large number of plant toxins are capable of producing hepatic necrosis, cirrhosis or carcinoma in animals. Many authors have suggested that these might be of significance in human cases, and the following outline is far from exhaustive. While it may have been proved that some of these substances are capable of producing liver damage in man, there is at present no epidemiologic evidence that any are causally related to human hepatomas:

(i) *Pyrollizidine alkaloids.* The experimental production of hepatomas by the hepatotoxic alkaloids of the genus *Senecio* led Schoental and Magee[42] to suspect that these substances might be important in human liver cancer. There is no apparent association between liver cancer and seneciosis in Africa, either in case histories or from the geographical distribution of these two diseases. The pyrollizidine alkaloids, however, are found in a very wide range of unrelated plant genera which are widely distributed throughout the world. Only 10% of the 2 000 species of these genera have been examined chemically,[43] so that exposure to these alkaloids might easily be unsuspected.

(ii) *Capsicum spp.* Hepatomas have been produced in rats with Chili peppers at a level of 10%.[44] The diet was choline-deficient, as Hoch-Ligeti points out, and hepatomas were prevented by feeding choline or by riboflavin deficiency. Peppers are relatively seldom used in South Africa.

(iii) *Tannic acid.* Tannic acid is hepatotoxic and can cause carcinoma of the liver experimentally,[45] but there is no reason to regard it as relevant in Africa.

(iv) *Cycadaceae.* The finding of a naturally-occurring carcinogen in the nut of a plant used by the people of Guam as a source of starch is most interesting, especially in view of the impression that the death rates from cirrhosis and primary liver cancer among the Guamanians are somewhat higher than those of the continental USA.

In 1963, Laqueur *et al.*[46] demonstrated that feeding of cycad nut meal (*Cycas circinalis L*) to rats gave rise to tumours in liver and kidneys, lung and intestine. The liver tumours were hepatocellular hepatomas and reticulo-endothelial neoplasms. They emphasized the similarity between the aglycone of a glycoside, cycasin, isolated from this species, and the chemical structure of dimethylnitrosamine. Two or three feedings of 5% cycad meal gave hepatomas in guinea-pigs.[47] This is particularly impressive in view of the resistance of guinea-pigs to carcinogens, including the amino-azo-dyes and aminofluorene—though not to dimethylnitrosamine.

The toxic aglycone appears to be methylazoxymethanol.[48] The view that it is the aglycone that is carcinogenic has been confirmed by Laqueur who showed that the cycad meal and cycasin (methylazoxy-

methanol glycoside) were unable to produce liver damage in germ-free rats. Evidently the β-glucosidase necessary for splitting the glycoside is present in the intestinal flora.[49] Further studies by O'Gara *et al.*[50] into the use of the emulsion as a topical dressing for skin ulcers, have shown that aqueous emulsions of the cycad nut applied to ulcers induced with croton oil in mice were also able to induce tumours of the liver and kidneys over 1 year later. The emulsion appeared to promote healing of the ulcers.

The family Cycadacea has many representatives in Africa. *C. circinalis L.* subsp. *madagascariensis* Schuster is eaten in times of famine by the population of Tanganyika, as is also the ripe seed of *Encephalartos spp.* by Zulus. Toxic effects have been reported from some of these.[51] These findings have not been put to the test in Africa, but the distribution of cycads[52] would make this source of limited carcinogenic significance, since species of this family are not found in Senegal. Liver cancer in the large cities would also be difficult to explain.

(v) *Dioscorea spp.* Gilbert and Gillman reported massive necrosis of the liver with post-necrotic scarring and nodular hyperplasia in weanling rats fed on yam (*Dioscorea rotunda*):[53] this was not produced in adult rats. They were not able to decide whether the lesion was the consequence of a deficiency or of a specific toxic principle. Steyn[54] has published the results of an investigation of cases of fatal accidental poisoning in Africans in Northern Rhodesia, and concluded that it was due to eating in the raw state a number of toxic plants, including *Dioscorea quartiniana* (mistaken for the non-toxic *D. schimperiana*), or certain toxic plants collected for use as relishes (spinach) e.g. *Acalypha indica* mistaken for *Amaranthus spp.*, which may, however, also be dangerously toxic (nitrates or cyanides). Only adult females and children of both sexes under 16 years of age were affected—which makes this type of poisoning seem unlikely to be of importance in liver cancer which affects males more than females.

(vi) *Crotalaria fulva.* Bras[55] and others have demonstrated that cirrhosis in children and adults in the West Indies may result from the ingestion of bush teas. There is no evidence of tumours being produced with these and the low incidence of primary liver cell carcinoma is stressed by Bras, Brooks and De Pass.[56]

(vii) *Other.* Neame and Pillay,[57] investigating hypoglycaemia in African patients, noted that some did not respond to intravenous dextrose, and death occurred soon after admission, being accompanied by an acute diffuse centrilobular zonal necrosis of the liver, and, in many, bilateral acute tubular necrosis. Most of the patients had taken African herbal medicines. Such medicine, obtained from the relatives of two sisters who had died following its administration, was injected intraperitoneally in rats, which died with typical lesions of the liver and kidney.

Several authors have suggested that the use of toxic herbs might lead to cirrhosis of the post-necrotic type,[57,58] and primary carcinoma of the liver. Prates has also suggested that herbal remedies may be responsible.[34]

It is noteworthy that Grusin mentions that potassium dichromate may be mixed with herbs, so that the blame for the lesions in his cases may not necessarily be easy to apportion to a particular ingredient of the mixture.

12. *Infectious Agents*

The relative immunity of Whites and Indians living in close association with the Bantu and Coloureds would make an infectious agent, or one present in air or water, seem unlikely.

(a) *Viruses.* The association with post-necrotic cirrhosis led Higginson and Steiner[5] to suspect infectious hepatitis in the Johannesburg material. In Uganda, Steiner and Davies[10] also suspected a viral hepatitis, although here post-necrotic cirrhosis was rare and the evidence for a virus was the inflammatory reaction sometimes accompanied by liver cell damage. There is no evidence that infectious hepatitis is excessively common in the Bantu. In Egypt, where it is endemic, liver cancer is rare.[59] In the absence of a satisfactory test for a previous exposure, this hypothesis remains speculation.

(b) *Parasites.* Possibly by analogy with the cholangiocellular carcinomas of the larger bile ducts caused by infection with *Clonorchis sinensis*, there has been a long-standing suspicion that bilharziasis might be responsible for the African disease. The high incidence of bilharziasis in Lourenco Marques led Prates to consider it to be causal.[60] This association could not be confirmed in Johannesburg in a series of cases of liver cancer matched with controls at postmortem examination.[61] Furthermore, bilharziasis is rare in French West Africa where liver cancer is frequent, while liver cancer is rare in Egypt where bilharziasis is frequent. Recent studies in Lourenco Marques have shown that the previous association was an artefact of diagnostic stringency, for more cases with liver cancer came to postmortem. When postmortem cases were matched, no correlation was discernible.[7]

THE MYCOTOXIN HYPOTHESIS

> 'Hear this, O ye that swallow up the needy, even to make the poor of the land to fail . . . making the ephah small, and the shekel great, and falsifying the balances by deceit . . . yea, and sell the refuse of the wheat.'
>
> Amos[62]

Although all natural hepatotoxins are suspect,[43] the hypothesis which best fits the epidemiologic evidence at present is that which attributes the high incidence of liver cancer in Africa to the ingestion of food contaminated with hepatotoxic substances from moulds.

The existence of mycotoxins has been recognized as long as poisonous fungi have been known. Although the *Oxford English Dictionary* is silent as to the origin, it would seem reasonable to derive the word 'toadstool' from *tod*, the Anglo-Saxon for 'death'. Liver damage is a common consequence of toadstool poisoning, and the Death-cap, *Amanita phalloides*, produces amanitine and phalloidine which are both hepatotoxic.[51]

Ethyl alcohol is an even more familiar example of a fungus-produced toxin associated with liver damage, but it seems to be palpably artificial to put alcohol in the same class as these other exceedingly potent substances.

Evidence that mouldy food may be dangerous was forthcoming in this country during the last century. In the 1890s a ship arrived in Port Elizabeth with a cargo of rice,[63] part of which was spoilt by sea water, and, being mouldy, was sold at Armstrong's Auction Rooms, Main Street, Port Elizabeth. Shortly afterwards it was reported that a large number of pigs fed with this rice had mysteriously died. The deaths were attributed to the mouldy condition of the rice.

Since then a number of obscure liver diseases in animals have been traced to the rations and, in due course, to mouldiness in some ingredient. Thus, 'hepatitis X' was reported in dogs in 1952. In 1955 this disease was attributed to a commercial dog feed, and in 1959 was produced by feeding with mouldy corn, poisonous for pigs.[64] Mouldy corn poisoning was described in pigs in 1953 and was reproduced in 1957 with pure cultures of the moulds *Penicillium rubrum* and *A. flavus*. These were both toxic to cattle and mice, while *P. rubrum* was also poisonous to the horse and goat.[65]

The carcinogenicity of hepatotoxins derived from moulds was first shown in Japan, where the toxicity of yellow rice was found to be due to contamination with *P. islandicum* Sopp. This fungus produces two hepatotoxic compounds—a chlorine-containing peptide and luteoskyrin, the latter capable of inducing carcinoma of the liver experimentally.[66] Despite such evidence of liver damage by mould metabolites the wider significance of these facts was not generally recognized until a mysterious disease of turkeys in Great Britain in 1960 had been traced to the inclusion in the feed of groundnut meal contaminated with *A. flavus*, which produced specific hepatotoxic substances.[67] The inclusion of this meal in purified diets gave rise to liver tumours in rats.[68]

The toxins produced by certain strains of this mould are termed aflatoxins, of which 4 related chemical fractions have been isolated. These cause liver damage in a large number of species of animals, and induce hepatomas in rats, ducks and trout. Aflatoxin is, in fact, the most active hepatocarcinogen known, being 1,000 times more potent than the azo dyes commonly used in experimental production of liver cancer. Short-term administration of as little as 10 μg. per day is sufficient to give nearly 100% incidence of liver tumours in rats.[69] In rats, hepatomas may arise without any evident intervening liver damage, and have been produced on continuous feeding with doses as low as 0·4 p.p.m.[70]

The importance of this source of hepatotoxins was generally recognized following these publications. Mycotoxicosis explained mysterious outbreaks of liver disease which had been known to occur in guinea-pigs fed on certain batches of commercial pelleted Diet 18. Schoental[71] found that a toxic batch of this diet would produce liver carcinomas in rats, and attributed this to contamination of the groundnut component (which formed 15% of this diet) with *A. flavus*. It explained also the findings in Morocco of an outbreak of liver tumours in pigs that since 1944 had been fed exclusively on oil presscake. Ninard and Hintermann[72] observed that only mechanically prepared presscake was responsible, and that which had been exposed to solvent extraction was innocuous. These tumours were associated with fatty degeneration, cirrhosis and hyperplastic nodules, and were produced with cake produced from cottonseed, palm and karité nuts. From this they deduced the possible importance of vegetable toxins in causing liver cancer, and referred to the use of karité-butter by the Senegalese as a possible cause of liver cancer in man there. Mycotoxicosis was not considered, but it may be significant that aflatoxins are fat-soluble and might have been removed by the solvent extraction.

Finally the question was considered whether mycotoxicosis could explain liver cancer in man in Africa. To assign credit for this hypothesis is difficult, and perhaps futile. Liver cancer in Africa has been a puzzle over many years, and current hypotheses have carried little conviction. The discovery of this unsuspected series of carcinogens illuminated many puzzling features of the human disease. In 1960 the Japanese workers[66] commented on the geographical pathology of primary carcinoma of the liver, and noted the importance generally attached to the nutritional factor, but commented 'there remains a question from a toxicological standpoint'. To my knowledge the first clear suggestion that these toxins might explain liver cancer in Africa, was published by Le Breton, Frayssinet and Boy[73] in 1962, but the same idea must have struck many others independently.[4,74,75] With the present facility of communication, priority in an idea is largely becoming a technicality, subject to accidents of publication.

A considerable range of other mould genera and species have been shown to produce toxins,[76] and among these, *A. ochraceus* produces a newly identified group of metabolites, ochratoxins, which are as toxic as aflatoxin.[77]

The following indirect evidence supports the view that a high risk of liver cancer is associated with greater exposure to mouldy foodstuffs:

1. *Stored Food exposed to High Relative Humidity*

A high relative humidity is necessary for mould growth, e.g. *A. flavus* requires a relative atmospheric humidity of over 80%. Areas of high liver cancer incidence in Africa are all regions of high humidity. Low rates occur in populations of dry regions such as Egypt, or in humid areas where food is not stored long enough for mouldiness to develop. An example of the last group is provided by the Ganda in Uganda, whose carbohydrate intake consists mainly of *matoke* (fresh steamed plantain) which has no chance to become mouldy.

A survey of cancer in the South African Lowveld has also provided striking examples of this association. Here a high frequency occurs in the rural farming areas of the Southern Lowveld (Barberton, Shongwe and Nelspruit hospitals) which are more humid than those of the Northern Lowveld (Acornhoek, Meetsa-a-Bophela and Shiluvane hospitals) where the relative frequency of liver cancer is lower. The hospitals in or adjoining the neighbouring mist-belt, however, (Sabie, Pilgrim's Rest, Bourke's Luck and Masana) also show a comparatively low liver cancer incidence, although the rainfall exceeds 50 inches per year. These are areas of afforestation, where the bulk of the food is purchased, and presumably far less susceptible to mycotoxin contamination.

2. *Food Storage provides Opportunities for Mould Contamination*

Contamination with spores and subsequent growth of mould may occur with modern procedures of harvesting and storage, but this is much more likely with primitive agricultural methods. It may take place before harvesting (especially as a result of insect infestation), during harvesting, during subsequent treatment or during storage.

Details of indigenous grain storage practices have been provided by Prof. M. J. Oosthuizen, who has also kindly permitted the reproduction of some illustrations of methods employed in Southern Africa (Figs. 1 - 4). Here grain is stored in pits, clay pots, cylindrical cribs (made of poles plastered with mud), grain huts, grain baskets (buried, or stored above ground in huts, or in thatched granaries) and grain bags made of two ox-hides sewn together. More recently, jute bags and galvanized tanks have been used. Professor Oosthuizen confirms that pit storage is often associated with mouldiness and loss of germination capacity (probably due to overheating of moist grain).[78] Subsequent access of moisture and termites may cause further damage. In such primitive stores, grain may be kept for up to 5 years and still be considered fit

Fig. 1. Container for storing shelled maize.

Fig. 2. An underground grain pit (*khilitesi*) in Modjadji's location, Northern Transvaal. The pit is generally funnel-shaped with a capacity of 10 - 60 bags or more. [From Oosthuizen, M. J. (1944): *Farming in South Africa*, **19**, 376—by kind permission of the author and publisher.]

Fig. 3

Fig. 4

Figs. 3 and 4. A Native granary in Ovamboland, SWA. The largest grain basket has a capacity of 150 bags.

for human consumption. With this degree of exposure to moisture and mould contamination, combined with an absence of grading, it will be evident that, especially during poor seasons, food may be eaten which is not fit for man or beast. The degree of unfitness of what is consumed will depend on a multitude of influences, among which tribal customs may be of considerable significance.

In tropical Africa, manioc, or cassava (*Manihot utilissima* Pohl) is a crop of considerable importance in human nutrition, and is one which would seem peculiarly subject to contamination with moulds. Its cultivation requires a frost-free growing season, an average annual rainfall over 30 inches and an altitude below 4,000 feet. During preparation of the tuber, e.g. in soaking and drying, it is extremely liable to mouldiness. Patently mouldy material may be offered for sale (as I have seen in Kisumu, Kenya). Furthermore, not being particularly palatable, this foodstuff is widely stored for times of scarcity. The leading producers are Nigeria, Congo (Leopoldville), Rwanda, Burundi and Ghana, and this crop is also grown in the humid regions of West Africa, Kenya, Uganda, Malawi, Zambia and Mocambique, most of which are known to be regions of high liver cancer incidence.

CONCLUSIONS

The mycotoxin hypothesis thus explains certain of the outstanding anomalies of the liver cancer distribution in

Africa. The different risks observed in various ethnic groups may be accounted for on the known differences in harvesting and food-storage practices, the greater dependence of the poorer classes on starchy foods, as well as their higher risk of antecedent liver damage from other causes, which may render them that much more susceptible to mycotoxins.

Fluctuations in incidence may be explained on the fluctuations in the mycotoxin content of the diet. These may depend on periods of scarcity, which oblige groups to consume mouldy foodstuffs that would otherwise have been discarded, or on seasonal fluctuations in humidity which may cause large-scale contamination of the crop. This was seen in South Africa in 1963 in the widespread contamination of the groundnut crop, attributable to unseasonable humidity.[79] Certain qualifications of this hypothesis are needed:

1. No specific mycotoxin is yet implicated. Although investigators have tended to concentrate on *A. flavus* and its aflatoxins, a variety of other fungi are known to produce hepatotoxins.
2. No specific crop is implicated. The initial demonstration of mycotoxins in groundnuts does not imply that consumption of this food is a prerequisite for the development of liver cancer.
3. It is not known whether single acute exposures are responsible, or whether the summation of subliminal exposures is sufficient. The rising incidence with age is certainly consistent with a process of summation.

The hypothesis has, in addition, certain obvious defects. Foremost of these is the difficulty of disproof. Postulating, as it does, a past fortuitous exposure to a diet contaminated with a toxigenic strain, of unspecified but probably ubiquitous moulds, this theory is sufficiently elastic to fit almost any existing facts of liver cancer incidence. Secondly, it does not provide a simple explanation of the sex ratio in liver cancer in Africa. This may be accounted for by the greater intake of food by males or to sex differences in susceptibility. Other anomalies may be easier to explain. Thus Brazil, from which the original toxic groundnuts came, has not yet been shown to have a high liver cancer frequency. Apparently the groundnut crop is exported and is not consumed locally.

Finally, although this hypothesis fits the facts better than the previous hypotheses, the postulates required for proof of a causal relationship in a human cancer[80] have not all been met. It has still to be shown that the risk of liver cancer in a population rises in proportion with the degree of previous exposure to mycotoxins. This requires quantitative intra- as well as inter-regional comparisons of exposure and liver cancer risk. Unfortunately aflatoxin is very difficult to demonstrate in the liver. It could not be found 6 hours after feeding a rat with 1 mg. of aflatoxin, nor after continued feeding with 3 - 4 p.p.m,[69] so that some more indirect evidence of exposure will have to be sought. Improved control of mould contamination of foodstuffs may possibly be followed by corresponding changes in liver cancer incidence after allowance is made for the appropriate latent period (at present unknown).

The remaining requirements are satisfied. Aflatoxin has been demonstrated in foodstuffs in regions of high liver cancer incidence such as South Africa and Nigeria, and toxigenic strains of *Aspergilli* and *P. islandicum* have been isolated from cereals on the local market in Addis Ababa.[81] Although we do not have direct proof of hepatotoxicity in man, there is little doubt that the liver is exposed to ingested mycotoxins. Finally, experimental confirmation of the carcinogenicity of mycotoxins is abundantly available.

Recommendations

A detailed investigation of food storage habits, mycotoxin exposure and liver cancer risk is needed. Aflatoxin is not necessarily the major carcinogen involved, but its chemistry is well known, and techniques of estimation are reasonably simple. It might well be employed as an indicator of mycotoxicosis, just as benzo(a)pyrene is used as an index of the presence of a much wider range of carcinogenic hydrocarbons in studies of air pollution.

Some might ask if we need to look for further evidence. This attitude is exemplified by Government Notice No. 888 of 19 June 1964 which reads: 'No cereal, groundnut or groundnut product or other food intended for human consumption may contain Aflatoxin or other fungus-produced toxin.'[82]

As it stands, this regulation seems somewhat precipitate, for we still lack evidence that aflatoxin is dangerous to man, so that we have no proof as yet that application of this regulation would lead to the prevention of human disease, although this is highly probable.

While hygienic measures to improve the quality of food are always commendable, an acceptable threshold level of contamination is desirable. The widespread and too literal application of such a regulation might lead to the condemnation of foodstores which are desperately needed especially in areas of food scarcity, and could result in more deaths from hunger than the food itself might have caused from mycotoxicosis. On the other hand this should not deter sensible use of the powers which this regulation confers.

The problem is a tremendous one, affecting staple crops on which the nutrition and the economic stability of some countries depend. It involves nearly all the countries of Africa south of the Sahara, as well as China, Japan and Indonesia. The USA may also be implicated, for the incidence of primary carcinoma of the liver in both Whites and non-Whites in the USA[83] is many times higher than that of Denmark, though still well below that of the South African Bantu.[27]

It would seem sound at present to limit action in South Africa to general improvement of food storage and to institute widespread screening of all suspect foodstores, e.g. wheat and mealies as well as groundnuts. Those batches which are heavily contaminated should be condemned and the less contaminated should be diverted with circumspection to nutritional purposes where they are unlikely to cause harm.

This is no justification of laissez-faire, of which there is enough. The opposite error, equally beloved of politicians, is also unwarranted—that of instituting sweeping reforms before demonstrating their effectiveness and practicability in a pilot scheme. This, it may be remembered, was the

fallacy of the well-meant scheme for fortification of bread in South Africa.[84] Shotgun methods are usually wasteful of money and effort in dealing with specific agents. As is illustrated by species-sanitation in malaria control, specific agents can often be best attacked by specific measures, tailored to fit the requirements of the particular situation.

In this field of mycotoxicoses, there is sufficient ignorance as well as sufficient evidence to merit the substantial investment in research needed for an informed decision. If this hypothesis is confirmed it promises a tremendous reward—the prevention of one of the commonest cancers in Africa.

SUMMARY

Earlier hypotheses regarding the cause of liver cancer in Africa fail to explain the epidemiologic pattern of this disease, notably its rarity in Egypt. The hypothesis of mycotoxicosis resulting from spoilage of food by toxic moulds fits the distribution better, in that it accounts for the rarity of liver cancer in dry areas, where mould spoilage is minimal, or in tribes, e.g. in Uganda, which consume a predominantly fresh diet.

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REFERENCES

1. Ramalingaswami, V. (1964): Nature (Lond.), **201**, 546.
2. Oettlé, A. G. (1956): J. Nat. Cancer Inst., **17**, 249.
3. Southam, C. M. (1963): Cancer Res., **23**, 1105.
4. Oettlé, A. G. (1964): J. Nat. Cancer Inst., **33**, 383.
5. Platt, J. R. (1964): Science, **146**, 347.
6. Isaacson, C., Seftel, H. C., Keeley, K. J. and Bothwell, T. H. (1961): J. Lab. Clin. Med., **58**, 845.
7. Prates, M. D. and Torres, F. O. (1965): To be published.
8. Higginson, J. and Steiner, P. E. (1961): Acta Un. int. Cancr., **17**, 654.
9. Miyai, K. and Reubner, B. H. (1963): Arch. Path., **75**, 609.
10. Steiner, P. E. and Davies, J. N. P. (1957): Brit. J. Cancer, **11**, 523.
11. Quisenberry, W. B. (1960): Ann. N.Y. Acad. Sci., **84**, 795.
12. Maltoni, C. and Prodi, G. (1959): Acta Un. int. Cancr., **15**, 191.
13. Bersohn, I., Wayburne, S., Hirsch, H. and Sussman, C. D. (1954): S. Afr. J. Clin. Sci., **5**, 35.
14. South African Institute for Medical Research (1953): *Annual Report for the year 1953*, p. 20. Johannesburg: SAIMR.
15. Berman, C. (1951): *Primary Carcinoma of the Liver*, p. 164. London: H. K. Lewis.
16. Oettlé, A. G. and Higginson, J. (1965): To be published.
17. Davies, J. N. P. *in* Collins, D. H., ed. (1959): *Modern Trends in Pathology*, p. 334, London: Butterworth.
18. *Idem* (1952): W. Afr. Med. J., **1**, 141.
19. Knowelden, J. (1963): Proc. Roy. Soc. Med., **56**, 529.
20. Thomson, J. G. (1961): Acta Un. int. Cancr., **17**, 632.
21. Oettlé, A. G.: Unpublished data.
22. Brygoo, E. R. (1961): Acta Un. int. Cancr., **17**, 711.
23. Ratnoff, O. D. and Mirick, G. S. (1949): Johns Hopk. Hosp. Bull., **84**, 507.
24. Mulay, A. S. and O'Gara, R. W. (1959): Proc. Soc. Exp. Biol. (N.Y.), **100**, 320.
25. Oettlé, A. G., De Meillon, B. and Lazer, B. (1959): Acta Un. int. Cancr., **15**, 200.
26. Andervont, H. B. (1958): J. Nat. Cancer Inst., **20**, 431.
27. Higginson, J. and Oettlé, A. G. (1960): *Ibid.*, **24**, 589.
28. Des Ligneris, M. (1936): S. Afr. Med. J., **10**, 478.
29. Higginson, J. and Oettlé, A. G. (1957): Acta Un. int. Cancr., **13**, 601.
30. Denoix, P. F., Schlumberger, J. R., Laurent, C. and Maujol, L. (1957): *Monographie de l'Institut National d'Hygiene*, No. 12, p. 179. Paris: Ministère de la Santé Publique.
31. Bloomberg, B. M., Miller, K., Keeley, K. J. and Higginson, J. (1958): J. Endocr., **17**, 182.
32. Walker, A. R. P. and Arvidsson, U. B. (1953): Trans. Roy. Soc. Trop. Med. Hyg., **47**, 536.
33. Prates, M. D. (1940): S. Afr. Med. J., **14**, 95.
34. *Idem* (1957): Acta Un. int. Cancr., **13**, 662.
35. Edmondson, H. A. and Steiner, P. E. (1954): Cancer (Philad.), **7**, 462.
36. Kraybill, H. F. (1962): Clin. Pharmacol. Ther., **4**, 73.
37. Grusin, H. (1955): S. Afr. Med. J., **29**, 117.
38. Mertens, W. K. and Van Veen, A. G. (1933): Meded. Dienst. Volksgezondh. Ned.-Indië., **22**, 209.
39. Van Veen, A. G. and Mertens, W. K. (1933): Geneesk. T. Ned.-Ind., **73**, 1309.
40. *Idem* (1936): Arch. néerl. Physiol., **21**, 73.
41. Van Veen, A. G. (1950): Docum. néerl. indones. Morb. trop., **2**, 185.
42. Schoental, R. and Magee, P. N. (1959): J. Path. Bact., **78**, 471.
43. Schoental, R. (1963): Bull. Wld Hlth Org., **29**, 823.
44. Hoch-Ligeti, C. (1952): Tex. Rep. Biol. Med., **10**, 996.
45. Korpassy, B. (1960): *Abhandlungen der Deutschen Akademie der Wissenschaften zu Berlin: Klasse für Medizin*, no. 3, 18.
46. Laqueur, G. L., Mickelsen, O., Whiting, M. G. and Kurland, L. T. (1963): J. Nat. Cancer Inst., **31**, 919.
47. Spatz, M. (1964): Fed. Proc., **23**, 1384.
48. Kobayashi, A. and Matsumoto, H. (1964): *Ibid.*, **23**, 1354.
49. Laqueur, G. L. (1964): *Ibid.*, **23**, 1386.
50. O'Gara, R. W., Brown, J. M. and Whiting, M. G. (1964): *Ibid.*, **23**, 1383.
51. Watt, J. M. and Breyer-Brandwijk, M. G. (1962): *The Medicinal and Poisonous Plants of Southern and Eastern Africa*, 2nd ed., p. 1457. Edinburgh: E. & S. Livingstone.
52. Kurland, L. T. (1964): Fed. Proc., **23**, 1337.
53. Gilbert, C. and Gillman, J. (1963): Nature (Lond.), **198**, 196.
54. Steyn, D. G. (1965): S. Afr. Med. J., **39**, 344.
55. Bras, G. (1961): Fed. Proc., **20**, 353.
56. Bras, G., Brooks, S. E. H. and De Pass, E. E. (1955): Docum. Med. geogr. trop. (Amst.), **7**, 146.
57. Neame, P. B. and Pillay, V. K. G. (1964): S. Afr. Med. J., **38**, 729.
58. Gillman, J. and Gilbert, C. (1954): Ann. N.Y. Acad. Sci., **57**, 737.
59. Hashem, M., Zaki, S. A. and Hussein, M. (1961): J. Egypt. Med. Assoc., **44**, 579.
60. Prates, M.D. (1948): An. Inst. Med. trop. (Lisboa), **5**, 149.
61. Higginson, J. and De Meillon, B. (1955): Arch. Path., **60**, 341.
62. *Holy Bible* (authorised version), *Amos* 8:4-6.
63. Oettlé, E. F. (1965): Personal communication.
64. Bailey, W. S. and Groth, A. H. (1959): J. Amer. Vet. Med. Assoc., **134**, 514.
65. Burnside, J. E., Sippel, W. L., Forgacs, J., Carll, W. T., Atwood, M. B. and Doll, E. R. (1957): Amer. J. Vet. Res., **18**, 817.
66. Miyake, M., Saito, M., Enomoto, M., Shikata, T., Ishiko, T., Uraguchi, K., Sakai, F., Tatsuno, T., Tsukioka, M. and Sakai, Y. (1960): Acta path. jap., **10**, 75.
67. Sergeant, K., Sheridan, A., O'Kelly, J. and Carnaghan, R. B. A. (1961): Nature (Lond.), **192**, 1096.
68. Lancaster, M. C., Jenkins, F. P. and Philp, J. McL. (1961): *Ibid.*, **192**, 1095.
69. Butler, W. H.: Personal communication.
70. Butler, W. H. and Barnes, J. M. (1963): Brit. J. Cancer, **17**, 699.
71. Schoental, R. (1961): *Ibid.*, **15**, 812.
72. Ninard, B. and Hintermann, J. (1945): Bull. Inst. Hyg. Maroc, **5**, 49.
73. Le Breton, E., Frayssinet, C. and Boy, J. (1962): C.R. Acad. Sci. (Paris), **255**, 784.
74. Editorial (1963): J. Amer. Med. Assoc., **184**, 57.
75. Leading Article (1964): Brit. Med. J., **1**, 204.
76. Scott, D. B. (1965): Mycopathologia (Den Haag), **25**, 213.
77. Van der Merwe, K. J., Steyn, P. S., Fourie, L., Scott, D. B. and Theron, J. J. (1965): Nature (Lond.), **205**, 1112.
78. Oosthuizen, M. J. (1944): Farming in South Africa, **19**, 371.
79. Sellschop, J. P. F., Kriek, N. P. J. and Du Preez, J. C. G. (1965): S. Afr. Med. J., **39**, 771.
80. Oettlé, A. G. (1963): *Ibid.*, **37**, 935, 957 and 983.
81. Coady, A. (1964): Brit. Med. J., **1**, 1510.
82. State Department of Health (1964): Government Gazette, **12**, 9.
83. Dorn, H. F. and Cutler, S. J. (1959): *Morbidity from Cancer in the United States*. Publ. Hlth. Monogr. No. 56, p. 207. New York: US Department of Health, Education and Welfare.
84. National Nutrition Research Institute (1959): *Food Enrichment in South Africa*, CSIR Research Report, No. 172, p. 157. Pretoria: CSIR.