PARKLAND MEMORIAL HOSPITAL

MEDICAL GRAND ROUNDS

March 3, 1966

AFLATOXIN

Patient 1: [redacted]

The patient was a 67-year-old [redacted] man who was admitted to [redacted] on [redacted]/63 with complaints of abdominal swelling and diarrhea of five weeks' duration.

He had presumably been well until five months prior to his admission when he noted gradual onset of anorexia and weight loss which totalled approximately six and one-half pounds at the time of admission. The swelling of the abdomen appearing five weeks prior to admission was accompanied by cramping abdominal discomfort in the epigastrium and watery diarrhea five to six times a day. For two weeks prior to admission edema of both lower legs was noted.

The patient's past history was significant in that up until 12 years prior to his present illness he had been a consistently heavy drinker for many years consuming at least a fifth of a pint of 100% whiskey per day. During the 12 years prior to admission, however, drinking was confined only to four or five beers a day.

Physical examination showed a malnourished, edematous colored man. Spider angiomata over the chest, liver palms and gynecomastia were present. Examination of the abdomen revealed the liver to be enlarged 11 cm below the right costal margin, stony hard and suggestively nodular. The abdomen was markedly swollen. Extremities showed 3+ edema.

The laboratory examination was consistent with the ward diagnosis of Laennec's cirrhosis; total serum proteins were 8.2, albumin 3.2, globulin 5.0, thymol turbidity 16, cephalin flocculation 3+/3+, SGOT 95. It was of interest that in spite of anorexia and weight loss the patient's cholesterol was 463 mg.%.

Examination of the ascitic fluid revealed no malignant cells, however, a needle biopsy performed on [redacted]/63 demonstrated a hepatic carcinoma made up primarily of acinar cells arranged in cords. Bile duct proliferation was prominent and fibrosis was noted in the periportal areas indicating the presence of underlying Laennec's cirrhosis.

Studies were carried out on the cholesterol feedback system in the biopsy specimen obtained of this hepatoma with the following results:

Cholesterol in diet, 3 days	Cholesterol synthesis (μμmoles acetate-2-C^{14} per mg tissue)
Low (68 mg.)	76
High (3-4 gm.)	82
Low (68 mg.)	76

These data unequivocally demonstrate the absence of a normal cholesterol feedback system in the hepatoma.

The patient was treated with 5-fluorouricil during his hospital stay with little change in his course. He was discharged on [redacted]/63 but following an acute gastrointestinal hemorrhage was brought to the emergency room in a morbund condition on [redacted]/63.

Autopsy confirmed the previous diagnosis of protal cirrhosis with hepatocellular carcinoma with diffuse metastases throughout the liver and lungs. The immediate cause of death was hemorrhage from an ulcerated gastric ulcer.

In summary, the patient represents a long-standing alcoholic who developed portal cirrhosis. At age 67 a hepatic carcinoma developed in this cirrhotic liver leading to a rapid downhill course and death.

Patient 2: [redacted]

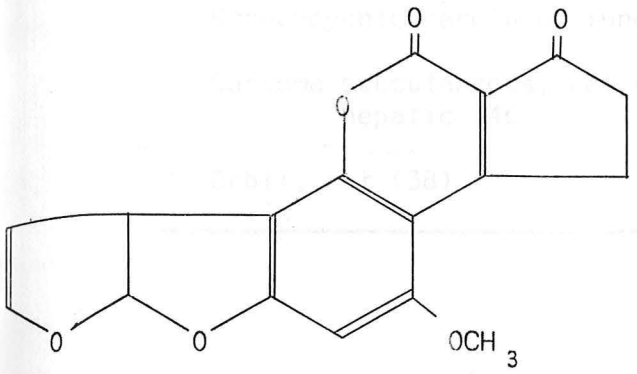
The patient is a 67 year-old white man whose last admission at [redacted] was from [redacted] to [redacted]66 with symptoms suggestive of severe angina.

The patient gives a past history of being in vigorous good health until 1937. He drank heavily and smoked at least one to two packs of cigarettes daily. At age 38 he had a myocardial infarction and since that time has suffered almost continuously from angina and severe peripheral vascular disease. In 1963 he developed symptoms of intermittent claudication and during an attempt to repairative vascular surgery a carcinoma of the colon was discovered. This lesion was ressected and the patient did well until 1965. At that time a primary carcinoma of the tongue developed and has been treated with radium implantation. During the same year the patient developed cough and hemoptysis; a primary bronchogenic carcinoma was discovered and has been treated with cobalt 60 external radiation.

Physical examination during the most recent admission shows only frequent premature contractions, a Grade II systolic murmur, numerous healed abdominal scars and a partial left hemiparesis.

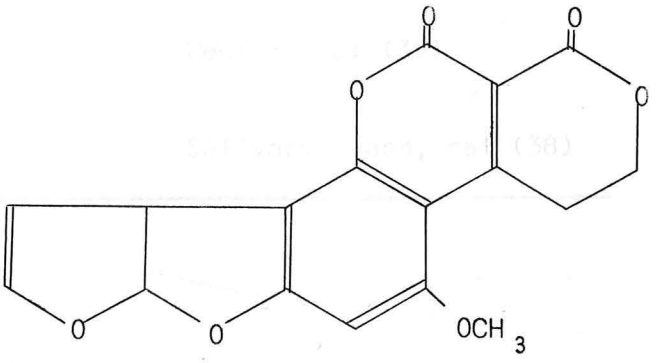
During the present admission the patient continued to have anginal pain but no evidence of recurrent myocardial infarction or carcinomatosis was noted and the patient was discharged five days after admission.

STRUCTURE OF AFLATOXINS



Aflatoxin B

I



Aflatoxin G

II

TABLE I

Species in which fatty liver and cirrhosis have been produced by aflatoxin

Turkey (11)	Cows (14)	Guinea Pig (34)	Goat (16)
Duck (12,13,22,23)	Cat (quoted in 44 reference 14)	Rat (21)	Pigs (15)
Chickens (12,13)	Dog (17,18)	Horses (16)	Monkey (19)
	Mice (16)		

TABLE 2

Tumors induced by aflatoxin

Hepatoma, Rat, Trout (33,34,35,36,37, 8,9,10)	Stomach, rat (38)
Bronchogenic carcinoma lung, rat (38)	Kidney, rat (38)
Sarcoma subcutaneous, rat (36) hepatic (46)	Rectum, rat (39)
Orbit, rat (38)	Salivary gland, rat (38)

TABLE 3

Grains and foods on which aflatoxin producing molds have been demonstrated

Aspergillus flavus

Peanuts	Corn	Wheat
Soybeans	Oats	Bermuda grass
Rice	Potatoes	Cotton meal

Penicillium islandicum

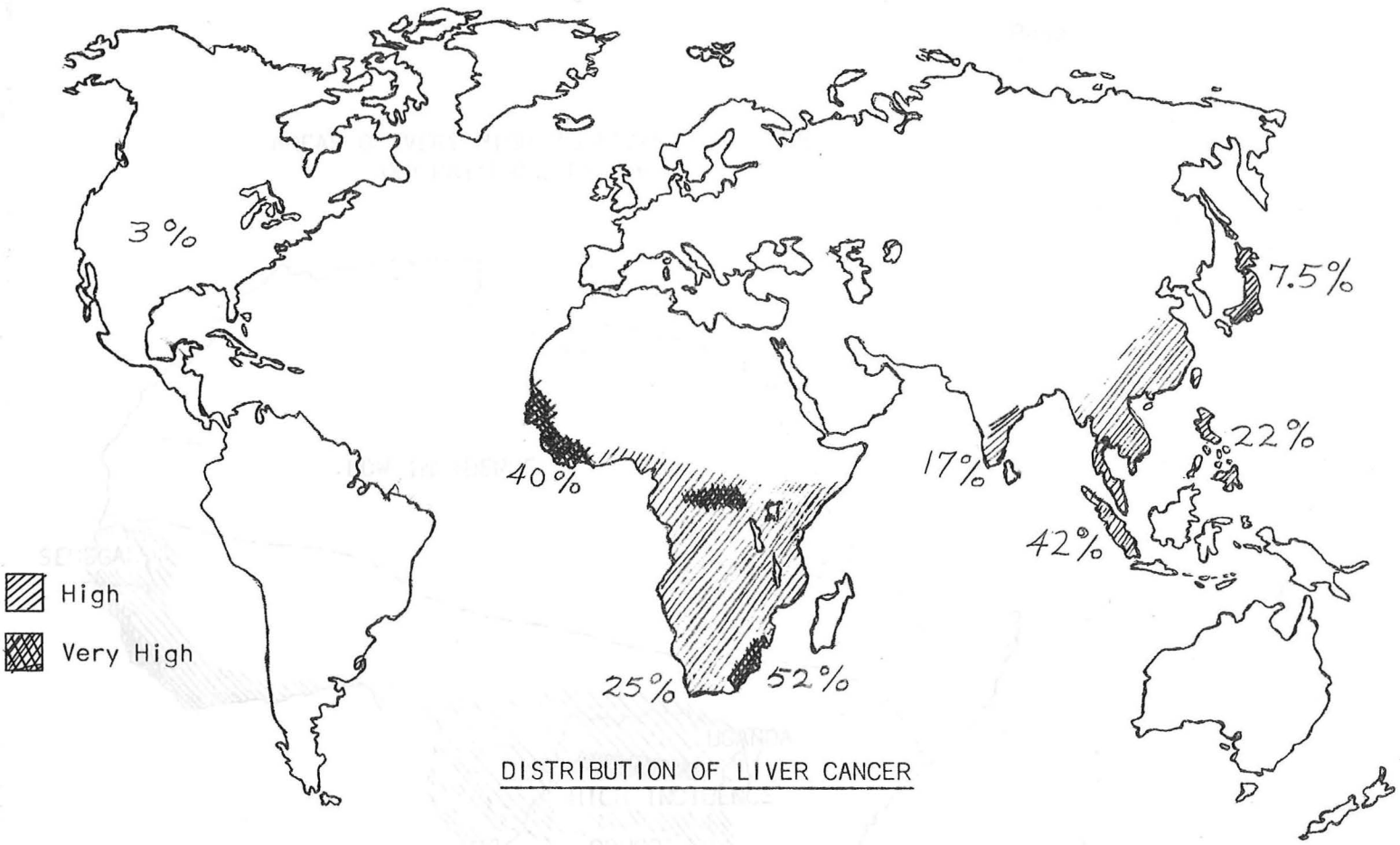
Rice

Penicillium rubrum

Corn

Penicillium puberulum

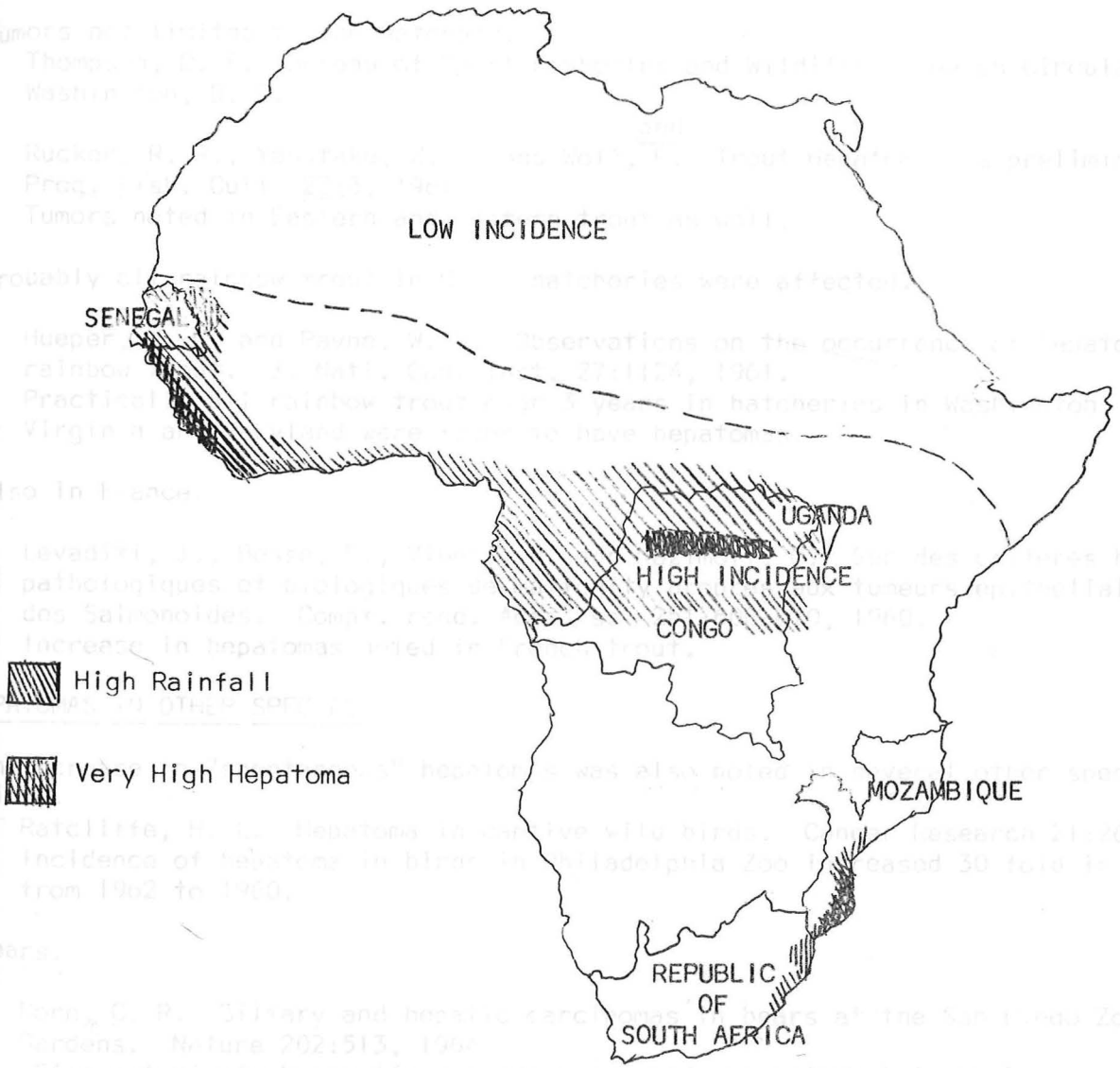
Potatoes	Wheat



DISTRIBUTION OF LIVER CANCER

DISTRIBUTION OF KWASHIORKOR

AREAS OF VERY HIGH HEPATOMA INCIDENCE
AND RAINFALL IN AFRICA



REFERENCES

TROUT HEPATOMAS

In April, 1960 hepatomas were noted in trout raised in Idaho.

1. Wood, E. M. Hepatic carcinoma in rainbow trout. Arch. Path. 71:471, 1961.
The first report of hepatomas appearing in rainbow trout in the U. S.

Tumors not limited to one hatchery.

2. Thompson, D. E. Bureau of Sport Fisheries and Wildlife Research Circular 10:1, 1960, Washington, D. C.

3. Rucker, R. R., Yasutake, W. T. and Wolf, H. Trout Hepatoma - a preliminary report. Prog. Fish. Cult. 23:3, 1961.
Tumors noted in Eastern and Western trout as well.

Probably all rainbow trout in U. S. hatcheries were affected.

4. Hueper, W. C. and Payne, W. W. Observations on the occurrence of hepatomas in rainbow trout. J. Natl. Can. Inst. 27:1124, 1961.
Practically all rainbow trout over 3 years in hatcheries in Washington, Idaho, West Virginia and Maryland were found to have hepatomas.

Also in France.

5. Levaditi, J., Besse, P., Vibert, R. and Nazimoff, O. Sur des criteres histo-pathologiques et biologiques de malignity propres aux tumeurs epitheliales hepatiques des Salmonoides. Compt. rend. Acad. sc. 251:608-610, 1960.
Increase in hepatomas noted in French trout.

HEPATOMAS IN OTHER SPECIES

An increase in "spontaneous" hepatomas was also noted in several other species
Birds.

6. Ratcliffe, H. L. Hepatoma in captive wild birds. Cancer Research 21:26, 1961.
Incidence of hepatoma in birds in Philadelphia Zoo increased 30 fold in autopsies from 1962 to 1960.

Bears.

7. Dorn, C. R. Biliary and hepatic carcinomas in bears at the San Diego Zoological Gardens. Nature 202:513, 1964.
Five out of six bears (four sloth bears and one grizzly) in the same area all died of liver tumors in a period of 6 years.

ETIOLOGY OF TROUT HEPATOMA

Cause of trout hepatoma traced to cotton meal.

8. Wolf, H. and Jackson, E. W. Hepatomas in rainbow trout: descriptive and experimental epidemiology. Science 142:676, 1963.
First evidence that cotton meal in dry trout ration was responsible for hepatomas.

Aflatoxin is implicated as cause of hepatomas.

9. Ashley, L. M., Halver, J. E., and Wogan, G. N. Hepatoma and Aflatoxicosis in Trout. Fed. Proc. 23:105, 1964.
Crude Aflatoxin fed to trout in a dose of 80 p.p.b. causes hepatomas.

Crystalline aflatoxin proven to be responsible for trout hepatomas.

10. Ashley, L. M., Halver, J. E., Gardner, W. K. and Wogan, G. N. Crystalline aflatoxins cause trout hepatoma. Fed. Proc. 24:627, 1965.
Crystalline aflatoxin B-1 produced hepatomas in 5 months. Approximately 20 mg given to a 200 gm trout causes acute hepatic toxicity.

TURKEY X DISEASE (or acute aflatoxin poisoning)

In Turkeys.

11. Stevens, A. J., Saunders, C. N., Spence, J. B. and Newnham, A. G. Investigation into disease of turkey poults. Vet. Rec. 72:627, 1960.
First clinical description of Turkey X disease.

Ducks and chickens.

12. Asplin, F. D. and Carnaghan, R.B.A. The toxicity of certain groundnut meals for poultry with specia. reference to their effect on ducklings and chickens. The Vet. Record. 73:1215, 1961.
Ducks and chickens also get "Turkey X" disease. Trial feedings implicate Brazilian peanuts as the source of the toxin.

and

13. Allcroft, R. and Carnaghan, R.B.A. Toxic Products of Groundnuts (peanuts). Chem. and Ind. January 12, 1963, page 50.
Reviews much of the unpublished early observations on "groundnut poisoning" in England, Spain and Hungary.

Cattle.

14. Loosmore, R. M. and Markson, L. M. Poisoning of cattle by Brazilian groundnut meal. The Vet. Record 73:813, 1961.
The first evidence that cattle can develop cirrhosis and fatty liver from peanuts.

Pigs.

15. Loosmore, R. M. and Harding, J.D.J. A toxic factor in Brazilian groundnut causing liver damage in pigs. The Vet. Record 73:1362, 1961.
First evidence that "Turkey X" disease could affect mammals, and here produces typical fatty liver and portal cirrhosis.

Horses, mice.

16. Burnside, J. E., Sippel, W. L., Forgacs, J., Carll, W. T., Atwood, M. B. and Doll, E. R. A disease of swine and cattle caused by eating moldy corn II. Experimental production with pure cultures of molds. Amer. J. Vet. Res. 18:817, 1957.
A. Flavius and P. rubrum grow on corn and are responsible for "moldy corn disease" in cattle. Swine, mice, horse all develop liver damage and jaundice.

Dogs.

17. Newberne, J. W., Bailey, W. S. and Seibold, H. R. Notes on a recent outbreak and Experimental Reproduction of Hepatitis X in Dogs. J. Am. Vet. Assoc. 127:59, 1955.
Three "epidemics" of a disease characterized by jaundice, fatty liver proceding to cirrhosis in dogs of the southeastern United States is described. The disease is reproduced by feeding either of two lots of dry dog food.

and

18. Bailey, W. S. and Groth, A. H. The relationship of Hepatitis X of dogs and moldy corn poisoning of swine. J. Am. Vet. Assoc. 134:514, 1959.
Corn contaminated with Aspergillus or Penicillium when fed to dogs will produce "Hepatitis X".

Primates.

19. Madhavan, T. V., Tulpule, P. G., Gopalan, C. Aflatoxin-induced hepatic fibrosis in rhesus monkeys. Arch. Path. 79:466, 1965.
I mg. of aflatoxin per day caused portal cirrhosis and death in monkeys in 32 days. This is probably the first experimental production of portal cirrhosis in a primate.

PURIFIED AFLATOXIN WILL REPRODUCE "GROUND NUT POISONING" IN DUCKS.

20. Butler, W. H. Acute liver injury in ducklings as a result of aflatoxin poisoning. J. Path. Bact. 88:189, 1964.
Careful description of sequential acute changes of oval cell proliferation in liver; produced with as little as 1.5 mg of aflatoxin.

And in rats.

21. Butler, W. H. Acute toxicity of aflatoxin in B_1 in rats. Brit. J. Cancer 18:756, 1964.
A single dose approximately 1.5 mg. of aflatoxin causes progressive biliary proliferation and collogen deposition which continues for at least one month.

CAUSE OF TURKEY X DISEASE TRACED TO ASPERGILLUS FLAVUS: ISOLATION AND CHEMICAL IDENTIFICATION OF AFLATOXIN

A toxic factor first noted in peanuts

22. Sargeant, K., O'Kelly, J., Carnaghan, R.B.A. and Allcroft, R. The assay of a toxic principle in certain groundnut meals. The Vet. Record 73:1219, 1961.
First successful preparation of a chloraform soluble factor from peanuts which would cause acute toxicity in ducks.

Aspergillus flavus was next implicated.

23. Sargeant, K., Sheridan, A., O'Kelly, J. and Carnaghan, R. B. A. Toxicity associated with certain samples of groundnuts. Nature 192:1096, 1961.
First noted that A. Flavus contaminated the poison nuts. A chloroform soluble, fluorescent material was responsible for Turkey X disease.

"Aflatoxin" purified in 3 separate laboratories.

24. Smith, R. H. and McKernan, W. Hepatotoxic action of chromatographically separated fractions of aspergillus flavus extracts. Nature 195:1301, 1962.
Isolation of five separate "aflatoxins" from A. flavus.

25. DeLongh, H., Beerthuis, R. K., Vles, R. O., Barrett, C. B. and Ord, W. O. Investigation of the factor in groundnut meal responsible for "turkey X disease". Biochim. Biophys. Acta 65:548, 1962.
Thin layer chromatographic isolation of four toxic compounds from A. flavus-molecular weight 312.

Crystalline Aflatoxin.

26. van der Zijden, A.S.M., Blanche Koelensmid, W.A.A., Boldingh, J., Barrett, C. B., Ord, W. O. and Philp, J. Isolation in crystalline form of a toxin responsible for Turkey X disease. Nature 195:1060, 1962.

and

27. Nesbitt, Brenda, O'Kelly, J., Sargeant, K. and Sheridan, A. Toxic Metabolites of Aspergillus flavus. Nature 195:1062, 1962.
The simultaneous isolation of crystalline "aflatoxin" by Unilever and Tropical Products Institute.

28. Sargeant, K., Carnaghan, R.B.A., and Allcroft, R. Aflatoxin: Chemistry and Origin. Chem. and Ind. January 12, 1963.
Review of early chemistry of isolation of aflatoxin.

structural studies.

29. van der Merwe, K. J., Fourie, L., and Scott, de B. On the structure of aflatoxins. Chem. and Ind. 1660, 1963.
First indication of structure of aflatoxins.

30. Bong Chang, S., Abdel Kader, M. M., Wick, E. L. and Wogan, G. N. Aflatoxin B_2: Chemical identity and biological activity. Science 142:1191, 1963.
Preliminary identification of structures of aflatoxin B_1 and B_2.

31. Asao, R., Büchi, G., Abdel-Kader, M. M., Chang, S. B., Wick, E. L. and Wogan, G. N. Aflatoxins B and G. J. Am. Chemical Soc. 85:1706, 1963.
The chemical differentiation of aflatoxin B and G by the MIT group.

Structures of Aflatoxins established.

32. Asao, T., Büchi, G., Abdel-Kader, M. M., Chang, S. B., Wick, E. L. and Wogan, G. N. The structures of aflatoxins B and G_1. J. Am. Chem. Soc. 87:882, 1965.
Definitive structural identification of aflatoxin B and G_1.

AFLATOXIN WILL ALSO PRODUCE TUMORS IN RATS AND PROBABLY IN ALL ORGANS

Rats - liver.

33. Lancaster, M. C., Jenkins, F. P. and Philp, J. McL. Toxicity associated with certain samples of groundnuts. Nature 192:1095, 1961.
Nine of eleven rats fed Brazilian peanuts developed hepatomas; two had pulmonary metastasis.

Rat - liver.

34. Schoental, R. Liver changes and primary liver tumours in rats given toxic guinea pig diet (M.R.C. diet 18) Brit. J. Cancer 15:812, 1961.
Evidence that (1) groundnuts in guinea pig diet are responsible for liver deaths in guinea pigs and (2) this meal fed for 12 months causes hepatomas in rats.

Rats - liver and kidney.

35. Salmon, W. D., and Newberne, P. M. Occurrence of hepatomas in rats fed diets containing peanut meal as a major source of protein. Cancer Res. 23:571, 1963.
Noted high incidence of "spontaneous" hepatoma in Charles River experimental rats. Traced to peanut meal. "U. S. Commerical". 15 of 88 rats had hepatomas in 1 year, 64/73 in 1-1/2 years. Kidney tumors in 16/73.

Rats - subcutaneous sarcoma and liver.

36. Dickens, F. and Jones, H.E.H. The carcinogenic action of aflatoxin after its subcutaneous injection in the rat. Brit. J. Cancer 17:691, 1963.
Rats injected with 50 mg or 500 mg aflatoxin twice per week. All developed sarcomas even on lower dosage.

Purified aflatoxin is responsible for the tumors produced by peanuts and is the most potent carcinogen so far discovered.

37. Barnes, J. M. and Butler, W. H. Carcinogenic activity of aflatoxin to rats. Nature 202:1016, 1964.
Purified aflatoxin 1.75 ppm will cause hepatomas in rats. Total dose 2.5 mg/rat.

Rat - lung, stomach, salivary gland, orbit.

38. Butler, W. H. and Barnes, J. M. Toxic effects of groundnut meal containing aflatoxin to rats and guinea-pigs. Brit. J. Cancer 17:699, 1963.
Rats fed 0.35 to 1.6 ppm aflatoxin for 26 weeks had hepatomas as well as primary tumors of the kidney, lung, stomach, salivary gland and orbit. Guinea pigs die in 3 weeks on this dose of aflatoxin.

Rat - stomach, rectum.

39. Barnes, J. M. Carcinoma of the glandular stomach in rats given diets containing aflatoxin. Nature 209:90, 1966.
Rats fed 3-4 ppm of aflatoxin for 3-104 weeks. Four carcinomas of the stomach and one of the rectum resulted.

TABLE 4

Relative carcinogenicity of aflatoxin

Agents	Species	Level/day to produce hepatomas
DAB (ρ - dimethylamino azobenzene)	Rat	3,000 µg.
ρ - dimethyl aminophenyl azo quinoline	Rat	1,000 µg.
aflatoxin	Rat	6 µg. (approx. 600 parts per billion in diet)
aflatoxin	Trout	0.005 µg. (0.5 parts per billion)

A. FLAVUS IS ONE OF THE COMMONEST MOLDS CONTAMINATING GRAINS.

Its growth characteristics and measures taken to control it are described in the following papers.

40. Austwick, P.K.C., and Ayerst, G. Groundnut microflora and toxicity. Chem. and Ind. January 12, 1963, page 55.
Reviews conditions needed for A. Flavus growth on nuts. 27 to 30% of peanuts have been shown to contain A. flavus. Mold grows both before harvesting and during prolonged drying. A. Flavus requires relatively high humidity of 70%.

41. Forgacs, J. and Carl, W. T. "Mycotoxicoses" in Advances in Veterinary Science, 1962, Academic Press, New York, page 273.
An excellent and thorough discussion of the many toxins produced by molds which cause disease in animals and man. All flour contains mold.

42. Graybill, H. F. and Shimkin, M. B. Carcinogenesis related to foods contaminated by processing and fungal metabolites. Adv. CAncer Res. 8:191, 1964.
Extensive review of entire mycotoxin problem.

43. Irving, G. W. USDA research program on mycotoxins. J. Am. Oil Chemists' Soc. 42:446A, 1965.
Surveys measures to detect and reject aflatoxin containing peanuts. Most peanuts with broken pods are contaminated with A. Flavus but less than 1% of "better quality" nuts have been rejected. Approximately 3% of "lower quality"nuts were rejected in 1964.

44. Spensley, P. C. Aflatoxin, the active principle in Turkey X disease. Endeavour 22:75, 1963.
A good general review of the agricultural problems of "groundnut" toxicity and Turkey X disease.

OTHER MOLDS THAN A. FLAVUS CAN PRODUCE AFLATOXIN OR HEPATITIC TOXINS

Penicillium puberulum.

45. Hodges, F. A., Zust, J. R., Smith, H. R., Nelson, A. A., Armbrecht, B. H. and Campbell, A. D. Mycotoxins: aflatoxin isolated from penicillium puberulum. Science 145:1439, 1964.
Penicillium puberulum will produce both aflatoxins B_1 and G_1.

Penicillium islandicum.

46. Kobayashi, Y., et al. Toxicological Studies on the Yellowed Rice by P. islandicum Sopp. III. Proc. Japan Acad. 35:501, 1959.
Experimental verification of primary hepatic carcinoma of rats by long-term feeding with fungus-containing-rice. Both hepatomas and sarcomas produced in rats fed rice contaminated only with P. islandicum.

47. Uraguchi, K., Tatsuno, T., Tsukioka, M., Sukai, Y., Sakai, M., Enomoto, M. and Kobayashi, Y. Toxicological approach to the metabolites of penicillium islandicum Sopp growing on the yellowed rice. Japan J. Exp. Med. 31:1, 1961.
Reviews the problem of "yellow" rice in Japan and demonstrates a hepatotoxin in P. islandicum contaminating yellow rice.

Penicillium rubrum

48. See reference 16.
P. rubrum probably produces the same toxin as A. Flavus.

AFLATOXINS ARE EXCRETED IN MILK AND ARE NOT DESTROYED BY PASTEURIZATION

49. de longh, H., Vles, R. O. and van Pelt, J. G. Milk of mammals fed an aflatoxin containing diet. Nature 202:466, 1964.
Cows fed aflatoxin excrete a toxic "milk factor" similar to but not identical with aflatoxin.

50. Allcraft, R., Rogers, H., Lewis, G., Nabney, J. and Best, P. E. Metabolism of aflatoxin in sheep: excretion of the "milk toxin". Nature 209:154, 1966.
Milk from sheep fed aflatoxin contain an aflatoxic metabolite which retains its toxicity.

MECHANISM OF AFLATOXIN CARCINOGENICITY

Inhibition of protein synthesis.

51. Smith, R. H. The influence of toxins of aspergillus flavus on the incorporation of [C14] leucine into proteins. Biochem. J. 88:50P, 1963.
Large doses of aflatoxin inhibits protein synthesis in liver slices.

Inhibition of RNA synthesis.

52. Clifford, J. I. and Rees, K. R. Aflatoxin: a site of action in the rat liver cell. Nature 209:312, 1966.
Aflatoxin binds directly to nuclear DNA and inhibits RNA synthesis and later - 15 minutes - protein synthesis.

Hepatomas characteristically lose the cholesterol feedback system.

53. Siperstein, M. D. and Fagan, V. M. Deletion of the cholesterol-negative feedback system in liver tumors. Cancer Research 24:1108, 1964.
Three hepatomas show loss with cholesterol feedback system.

54. Siperstein, M. D., Fagan, V. M. and MOrris, H. P. Further studies on the deletion of the cholesterol feedback system in hepatomas. Cancer Research 26:7, 1966.

55. Siperstein, M. D. Deletion of the cholesterol negative feedback system in precancerous liver. J. Clin. Invest. Abstract to be published.
Aflatoxin causes loss of the cholesterol feedback system.

56. Kellogg, K. K. Xanthomatosis in laying hens - a case report. Poultry Science 43:777, 1964.
Xanthomatosis is a very common disease in hens. Here traced to feed contaminated with A. flavus.

Aflatoxin will also interfer with metabolism in plants.

57. Schoenthal, R. and White, A. F. Aflatoxins and 'Albinism' in plants. Nature 205:57, 1965.
Aflatoxin completely blanches plants probably by stopping chlorophyl synthesis.

HUMAN HEPATOMAS IN THE UNITED STATES

There is evidence of an increasing incidence of hepatomas in the United States.

58. Steiner, P. E. Carcinoma of the liver in the United States.Acta Unio. Inter-Nationalis Contra Cancrum 13:628, 1957.
A general review of all available statistics.

59. Patton, R. B., and Horn, R. C. Primary liver cancer. Cancer 17:757, 1964.
Reviews the Henry Ford Hospital and previous literature. Concludes that the incidence in the United States is rising.

60. Benner, E. J. and Labby, D. H. Hepatoma: clinical experiences with a frequently bizarre tumor. Ann. Int. Med. 54:620, 1961. The Oregon University cases of hepatoma are reviewed. Briefly summarizes evidence that the incidence is increasing.

61. MacDonald, R. A. Primary carcinoma of liver, clinicopatholigic study of 108 cases. Arch. Int. Med. 99:266, 1957.
Boston City Hospital experience - the incidence of hepatomas has doubled from 1947-1954 as compared with previous 30 years.

HUMAN HEPATOMA INCIDENCE THROUGHOUT THE WORLD

Hepatoma is primarily a disease of the moist tropical areas of the world.

62. Berman, C. Primary carcinoma of the liver in Advances in CAncer Research 5:55, 1958.

and

63. Berman, C. Primary Carcinoma of the Liver H. K. Lewis, London, 1951.
Extensive reviews of the geographical distribution of hepatoma.

Africa

64. Oettle, A. G. Cancer in Africa, especially in regions south of the Sahara. J. Natl. Cancer Instit. 33:383, 1964.
Most extensive and balanced discussion of incidence of all cancers in Africa. Raises the possibility that A. flavus contamination may be responsible for high incidence of liver tumors.

Mozambique, Africa

65. Prates, M. D. Malignant Neoplasms in Mozambique. Brit. J. Cancer 12:177, 1958.
Carcinoma of the liver accounts for 43% of all tumors in African men in Mozambique and is twice as common as in South Africa.

West Africa

66. Findlay, G. M. Observations on primary liver carcinoma in West African soldiers. J. Royal Micro. Soc. 70:166, 1950.
70% of cancer deaths in West African Nigerian soldiers during World War II were due to hepatoma.

Indonesia

67. Bonne, C. Cancer in Java and Sumatra. Am. J. Cancer 25:811, 1935.
Hepatoma is the commonest cancer amongst both the Chinese and native Indonesians of Indonesia.

India

68. Reddy, D. G. and Rao, K. S. Primary carcinoma of the liver among South Indians. J. Indian Med. Assoc. 39:1, 1962.
One of the few studies of hepatoma in India. The incidence of hepatoma in southern India (Madras) is as high as in Africa. Liver tumors in northern India (Bombay) are less common.

Hong Kong and China

69. Hou, P. C., Schweiz, Z. Primary carcinoma of the liver in the community of Hong Kong. Path. Bakteriol 18:657, 1955.
35% of all cancers and 7% of all deaths in Chinese (Cantonese) in Hong Kong are due to hepatoma. Cirrhosis is seen in all malignant livers.

Malaya

70. Marsden, A.T.H. The Geographical Pathology of Cancer in Malaya. Brit. J. Cancer 12:161, 1958.
Incidence of hepatoma is high - 5% of Chinese and Malays in Malaya and in Singapore. There is no association with malnutrition.

Japan

71. Takeda, K. Cancer of the stomach in Japan from the viewpoint of Pathological Anatomy. Schweiz z Path u Bakt. 18:538, 1955.
Carcinoma of liver is about twice as common in Japan as in U. S.

72. See also reference 47.

STATE OF UNDERNUTRITION IN TROPICAL COUNTRIES HAS BEEN WELL DOCUMENTED

73. Berman, C. Nutritional states in the causation of primary liver cancer. Schweiz z Path u Bakt. 18:598, 1955.
An extensive report of an international survey of world nutritional states reviewed in regard to kwashiorkor and liver cancer. The author suggests that low protein diets may be responsible for both but clear cut disassociations in Jamaica, South and Central American - high kwashiorkor, low hepatoma; and in Singapore, Japan and Indochina and Philippines - low kwashiorkor and high hepatoma rates are present.
The main source of food throughout the tropics is rice or maize eaten usually after storage or purposely fermented, eg. rice cakes of Indonesia.

THE RELATIONSHIP OF MALNUTRITION AND KWASHIORKOR TO CIRRHOSIS AND HEPATOMA

There is no evidence that either starvation or a low protein diet causes cirrhosis in either animals or man.

74. Hartroft, W. S. in Rouiller's "The Liver" II, Acad. Press, New York, 1964, page 489.
Cirrhosis is not seen in rats who are starved.

75. Sherlock, S. and Walshe, V. Effect of undernutriton in man on hepatic structure and function. Nature 161:604, 1948.
Chronic undernutrition to the point of edema in a postwar German city did not produce cirrhosis in any of 21 civilians studied by liver biopsy.

There is good evidence that Kwashiorkor does not cause the cirrhosis.

76. Brock, J. F. Survey of the world situation on kwashiorkor. Ann. N. Y. Acad. Science 57:696, 1954.

and

77. Gillman, J. and Gillman, T. Perspectives in Human Nutrition. 1951, Grune and Stratton, New York.

and

78. Gillman and Gilbert. Aspects of nutritional liver disease - human and experimental. Ann. N. Y. Acad. Science 57:737, 1954.

These studies conclude that (1) kwashiorkor does not lead to the cirrhosis of the Bantu and (2) American kwashiorkor is not associated with cirrhosis.

Kwashiorkor is probably due solely to lack of protein in the child's diet.

79. Lowe, E. C., Pretorius, P. J., Daniel, JG.A. and Hendrickse, R. G. Kwashiorkor and protein malnutrition: a dietary therapeutic trial. Lancet 2:355, 1955.
Vitamin-free casein will reverse the signs and symptoms of kwashiorkor as well as will whole dry milk. Moreover same response can be produced with mixture of 18 amino acids plus vitamins.

80. Higginson, J., Grobbelaar, B. G. and Walker, A.R.P. Hepatic fibrosis and cirrhosis in man in relation to malnutrition. Am. J. Path. 3:329, 1957.
An excellent discussion of kwashiorkor and cirrhosis concluding that the two are unrelated diseases. The adult Bantu consumes 63 to 112 gms. protein per day; choline intake equals that of white population.

81. Higginson, J. Relation of carcinoma of the liver to cirrhosis, malaria, syphilis and parasitic diseases. Schweiz z Path. Bakt. 18:624, 1955.
Constant relationship between cirrhosis and hepatoma is clear. Siderosis in Africa is not related to either portal cirrhosis, and hepatomas are seen both in populations without siderosis (Uganda) and in those who don't drink (Moslems).

82. Higginson, J. The geographical pathology of primary liver cancer. Cancer Research 23: 1624, 1963.
Brief review of relationship between cirrhosis, kwashiorkor and hepatoma cancer, especially in Africa. Concludes that cirrhosis and hepatoma may have a common etiology but that kwashiorkor is unrelated.

83. Srikantia, S. G., Sriramachi, S. A followup study of fifteen cases of "Kwashiorkor". Ind. J. Med. Res. 46:121, 1958.
Fifteen cases of Kwashiorkor followed for up to 8 years with liver biopsies show no evidence of fibrosis.

84. Achar, S. T. Lecture VIII International Pediatric Conference, quoted in Reference 83. 17 cases of "K" followed with biopsies 4-8 years without cirrhosis.

MOLD CONTAMINATION IS A SIGNIFICANT PROBLEM, ACCIDENTAL OR INTENTIONAL, IN MOST OF THE GRAIN OF THE TROPICS

Over 30% of maize in South Africa is contaminated with A. flavus-oryzae.

85. National Nutrition Research Institute Annual Report, 1961-1962. South African Council for Scientific and Industrial Research Special Report 8:1, 1962.

"Yellow"rice in Japan

86. See Reference 47.

A flavus is intentionally used (in place of yeast) throughout the Orient to ferment rice (beer, sake, and rice cakes), barley (beer), and soy beans (soy sauce is made by prolonged fermentation with A. flavus).

87. Thom, C and Raper, K. B. "A Manual of the Aspergilli", Williams and Wilkins, 1945, pages 259, 269-271, and 249.

ALCOHOL AND CIRRHOSIS

The relation of cirrhosis to hepatoma is well established.

88. Hall, J. W. and Sun, S. C. Effect of portal cirrhosis on the development of carcinomas. Cancer 4:131, 1951.
Approximately 80% of liver carcinoma occurs in previously cirrhotic livers.

89. Levy, C. M., Gellene, R., and Ning, M. Primary Liver Cancer in Cirrhosis of the Alcoholic. Ann. N. Y. Acad. Scien. 114:1026, 1964.
84% of alcoholics with cirrhosis develop hepatomas, but author finds incidence of hepatoma 50% in severe cirrhotics.

Alcohol

90. Klatskin, G. Effect of alcohol on the liver. J.A.M.A. 170:119, 1959.
Excellent discussions of the relationship of alcohol to cirrhosis.

91. Klatskin, G. Alcohol and its relation to liver damage. Gastroenterology 41:443, 1961.
A balanced review of the relationship of alcoholism to cirrhosis, experimental and clinical.

Choline deficiency plus alcohol can cause liver necrosis and diffuse cirrhosis.

92. Lowry, J. V., Ashburn, L. L., Daft, F. S., Sebrel, W. H. Effect of alcohol in experimental liver cirrhosis. Quart. J. Stud. Alcohol 3:168, 1942.

93. Hartroft, W. S. "Experimental Cirrhosis in the Liver", Vol 2, Rouiller, C. Acad. Press, 1964.

But alcohol alone does not lead to cirrhosis in animals.

94. Moon, V. H. Experimental cirrhosis in relation to human cirrhosis. Arch. Path. 18:381, 1934.

95. Ashworth, C. T. Production of fatty infiltration of liver in rats by alcohol despite adequate diet. Proc. Soc. Exp. Biol. and Med. 66:382, 1947.

96. Rouiller, C. L. Experimental Toxic Injury of the Liver in "The Liver", Vol. 2, Acad. Press, 1964, page 335.
Complete review of all known hepatic toxins (aflatoxin is not mentioned).

Ethanol will cause a fatty liver.

97. Lieber, C. S. Hepatic and Metabolic Effects of Alcohol. Gastroenterology 50:119, 1966.

Complete review of ethanol induced fatty liver.

But there is no evidence that a fatty liver ever leads to cirrhosis.

98. Dible, J. H. Degeneration, Necrosis and Fibrosis of the Liver. Brit. Med. J. 1:883, 1951.
Discussion of weaknesses in experiments claiming to have produced cirrhosis in animals by nutritional means. Fatty liver in man or animals does not lead to cirrhosis.

There is no evidence that alcohol per se causes cirrhosis in man and pure ethanol does not aggravate established cirrhosis.

99. Erenoglu, E., Edreira, J. G. and Patek, A. J. Observations on patients with Laennec's cirrhosis receiving alcohol while on controlled diets. Ann. Int. Med. 60:814, 1964.
Twenty-two patients with moderately severe cirrhosis received 198 ml of absolute alcohol per day for two months. Eighteen improved and two showed some deterioration. Only one showed progressive cirrhosis.

100. Patek, A. J., Jr. and Post, J. Treatment of cirrhosis of the Liver by a nutriticus diet and supplements rich in vitamin B Complex. J. Clin. Inv. 20:481, 1941.
Four cirrhotics given nine ounces of 40% ethanol a day for 6 to 18 months showed no return of signs or symptoms of cirrhosis.

101. Summerskill, W.H.J., Wolfe, S. J., and Davidson, C. Response to Alcohol in Chronic Alcoholics with Liver Disease. Lancet 535:1957. Large doses of pure ethanol (90-120 ml 90%/day) caused increased appetite and body weight in established cirrhotics. Liver histology and function tests improved.

Conclude: "Alcohol may have a place in the treatment of liver disease"

POSSIBLE RELATION OF AFLATOXIN TO OTHER TUMORS

See References 36, 38, 39 and Table 2.

102. Terry, L. Smoking and Health - Report of the Advisory Committee to the Surgeon General of the Public Health Service. Public Health Service Publication No. 1103, page 148.

103. Wynder, E. L., Brass, I. J. and Feldman, R. M. A study of the etiological factors in cancer of the mouth. Cancer 10:1300, 1957.
Smoking is an important factor in development of cancer of the mouth (smoking increases the risk 3x); alcohol independently increases the risk 10x.