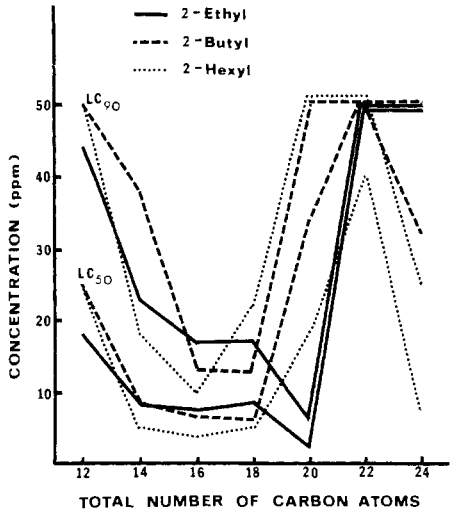

Figure 1. Structure–activity relationship of 2-substituted aliphatic carboxylic acids.

to the 3, 5, or 7 position in the hydrocarbon chain, the position of carboxyl substitution did not influence the activity markedly. For instance, 2-ethyltetradecanoic (3), 2-butyldodecanoic (10), and 2-hexyldecanoic (16) acids exhibited almost the same level of activity regardless of the position of the carboxyl group. However, being more active than its structural isomers, 2-butylhexadecanoic (12) and 2-hexyltetradecanoic (18) acids, 2-ethyloctadecanoic acid (5) was again an exception to this regularity.

Since the 2-substituted carboxylic acids are insoluble in water, they form a thin-layer film on the top of water in the custard dishes during the bioassay tests. It was reported that the activity of 3-methyloctadecanoic and 2,3-dimethyloctadecanoic acids could be enhanced manyfold by using emulsifiable preparations (Ikeshoji and Mulla, 1974b). It is, therefore, likely that the activity of the most active carboxylic acids studied here may be further enhanced by formulating them in suitable solvents and surface active agents.

LITERATURE CITED

Asinger, F., Berger, W., Fanghaenel, E., Mueller, K. R., *J. Prakt. Chem.* **22**, 153 (1963).
Breusch, F. L., Ulusoy, E., *Chem. Ber.* **86**, 688 (1953).
Hwang, Y.-S., Mulla, M. S., Arias, J. R., *J. Agr. Food Chem.* **22**, 400 (1974).
Ikeshoji, T., Mulla, M. S., *J. Econ. Entomol.* **63**, 90 (1970a).
Ikeshoji, T., Mulla, M. S., *J. Econ. Entomol.* **63**, 1737 (1970b).
Ikeshoji, T., Mulla, M. S., *Environ. Entomol.* **3**, 482 (1974a).
Ikeshoji, T., Mulla, M. S., *Environ. Entomol.* **3**, 487 (1974b).
Maw, M. G., *Nature (London)* **227**, 1154 (1970).
Nikishin, G. I., Ogibin, Y. N., Petrov, A. D., *Izv. Akad. Nauk SSSR, Otd. Khim. Nauk*, 1487 (1961).
Quraishi, M. S., *Can. Entomol.* **104**, 1499 (1972).
Quraishi, M. S., Thorsteinson, A. J., *J. Econ. Entomol.* **58**, 185 (1965).
Saxena, K. N., Thorsteinson, A. J., *J. Econ. Entomol.* **64**, 287 (1971).
Stanley, W. M., Jay, M. S., Adams, R., *J. Amer. Chem. Soc.* **51**, 1261 (1929).
Weitzel, G., Wojahn, J., *Hoppe-Seyler's Z. Physiol. Chem.* **285**, 220 (1950).

Received for review April 11, 1974. Accepted July 18, 1974.

Implication of Mycotoxins for Human Health

T. Colin Campbell[1] and Leonard Stoloff*[2]

The veterinary problem, Turkey X disease, led to the discovery of aflatoxins and to studies which demonstrated that toxins in the absence of visible molds could produce effects (carcinogenesis and organ damage) far removed in time from the cause. Only a few cases of acute mycotoxicoses in humans have been recorded. To shed some light on the risk to man from ingestion of aflatoxins in his food supply, published data on comparative metabolism, primate studies, inadvertant human incidents, and epidemiology are analyzed and presented in common terms to facilitate intercomparison. Although the data raise some questions, they presently provide the best available basis for estimating risk. The greatest attention has been given to aflatoxins, but other mycotoxins potentially capable of causing damage on chronic ingestion have also been considered. Among them are patulin, penicillic acid, trichothecenes, luteoskyrin, and citrinin.

The association of microbial toxins in foods or feed with human and animal disease has generally been based on symptoms occurring shortly after ingestion, providing a readily ascertainable cause-and-effect relationship. There are not many such situations involving molds. The first followed fast on the early developments in the field of microbiology. Ergot alkaloids and ergot poisoning, the first mold toxin and mycotoxicosis so recorded, were associated with growth of the mold *Claviceps purpurea* as early as 1711 (Barger, 1931). More than 200 years later two epidemic incidents occurred within a few years of each other. In Japan the "yellow rice disease," which caused many deaths, was associated with invasion of the rice by a number of molds including *Penicillium islandicum, P. citrinum,* and *P. citreo-viride* (Saito *et al.,* 1971; Uraguchi, 1971); in Russia, an even more severe loss of life was caused by ingestion of grain which had been invaded by *Fusarium tricinctum* (= *F. sporotrichiodes*) (Joffe, 1971).

Both incidents were caused by dislocation of normal food supplies due to wartime situations: rice from Southeast Asia stored under poor conditions was imported into Japan in unusually large quantities to make up for the Japanese production deficit; grain, which could not be harvested the previous fall because all available Russian manpower was at the fighting front, was salvaged from

[1] Department of Biochemistry and Nutrition, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061.
[2] Division of Food Technology, Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Washington, D. C. 20204.

the fields in the spring to avert starvation. The toxic agents produced by *P. islandicum, P. citrinum,* and *P. citreo-viride* were eventually isolated and characterized (Saito *et al.,* 1971; Uraguchi, 1971) but their natural occurrence in rice has not yet been demonstrated. The toxic agent involved in the Russian incident has not been adequately characterized, but from what little information is available on the isolated compounds, the implicated mold, and the symptoms of the illness, it is most probably one of the trichothecenes (Bamburg and Strong, 1971; Mirocha and Pathre, 1973). Neither incident aroused much concern, except for the people immediately involved.

In most societies, moldy foods, except for a few controlled fermentations, are rejected for aesthetic reasons, and moldy food has generally ended up as feed. Consequently, most of the original work and the current approaches on mycotoxicoses are based on the veterinary literature. Many incidents of animal mycotoxicoses must be unrecorded, but at least one incident was serious enough to have political impact. Khrushchev, in his memoirs (Khrushchev, 1970), records that in the tense period of 1939, just prior to World War II, horses were dying in epidemic proportions all over the Ukraine. At that time, horses were essential for farm work and military transport. The causative agent was eventually found to be a fungus, *Stachyobotris atra,* growing on wet hay (Forgacs, 1972). Toxins produced by a culture of *S. atra* have been recently characterized as trichothecenes (Eppley and Bailey, 1973). Another feed-related mycotoxicosis, occurring 21 years later (the so-called Turkey X disease), was also recorded (Lancaster *et al.,* 1961) but this time the findings made a major impact on the scientific community, culminating in the current concern about the possible effects of mycotoxins on human health.

Why should this latter veterinary problem, which principally involved turkeys and ducklings, have so much more impact than the human disasters? Part of the answer may be poor communication or intentional censorship about the human incidents; part of the answer may be the peculiar circumstances of each human incident, creating the feeling that these were acute manifestations of situations that, once described, could be easily avoided. Part of the answer is in the clever application to the feed incident of new and novel chemical and biological techniques to detect, isolate, and characterize the toxic agents in a short space of time and to make ample quantities of the toxins available for toxicological study. Most of the answer is probably in the evidence that was rapidly developed from the feed incident and subsequent experimentation that a common mold, *Aspergillus flavus,* could elaborate a potent carcinogen in a staple food, peanuts; that the carcinogen could be present in the absence of overt evidence of mold invasion; and that the potential existed for damage to the ingesting animal at a time far removed from the challenge. Part of the answer is in the decision made by the World Health Organization, Protein Advisory Committee, that peanuts could provide a cheap source of protein supplement to protein-deficient areas of the world and which now had to be critically examined in the light of the new finding (Protein Advisory Group, 1962, 1966).

Whatever the answer, in many countries those agencies responsible for the safety of the food supply reexamined their positions with regard to mold in food and feed. They considered the possibility that mold-elaborated toxins capable of causing damage upon chronic ingestion could be present in foods where no usual evidence of mold existed. At present, some type of guideline or regulation with regard to aflatoxin contamination of food and/or feed is in force in Brazil, Canada, Denmark, England, Germany, Hungary, India, Japan, The Netherlands, South Africa, and the United States. Strong research programs on mycotoxins are being pursued in Canada, Denmark, England, France, Germany, India, Japan, South Africa, and the United States. Now, after a decade of intensive and extensive research on the aflatoxins and an increasing spread of investigations to other toxic mold metabolites, what conclusions can be drawn about the implied dangers to human health?

First, the laboratory and field information confirm the historical evidence that levels of mycotoxins capable of immediate damage are not likely to be encountered in food. When mold has invaded a food to such extent that the amount of toxin is sufficient to cause an immediate response, the consumer will usually reject the food for aesthetic reasons, except in a starvation situation or in those few cultures where the staple food is intentionally molded. Even animals tend to reject heavily molded feed. When molded grain was used in studies of aflatoxicosis in farm animals, the animals would not accept toxic amounts of the grain unless it was sweetened with molasses (Armbrecht *et al.,* 1971). Many farmers will attest to the need of a sweetener to dispose of moldy grain in feed.

Investigations of delayed mycotoxin damage have chiefly concerned aflatoxins because of the rapid development of analytical techniques and the ready availability of toxin-producing cultures and isolated toxins in adequate quantities. Experiments with laboratory and domestic animals have demonstrated a wide difference in susceptibility to both immediate and delayed effects of the aflatoxins by sex, by different species (Wogan, 1968), and even by breeds (Gumbmann *et al.,*1970). Nutritional status is another possible predisposing factor (Madhavan *et al.,* 1965a,b; Rogers and Newberne, 1969, 1971). Understandably, the bulk of animal experimentation has involved susceptible animals and, in the absence of definitive data on man, conservative judgment on the risk to man must be based on the data from the most sensitive species. Disregarding the complications of breed, sex, and diet, the conservative judgment of risk is based on experiments (Wogan and Newberne, 1967) showing 100% incidence of hepatoma in rats on a lifetime diet containing aflatoxin B_1 at 15 μg/kg and a projection of those data to the possibility that a significant incidence of hepatoma will be found in rats on a diet containing 1 μg/kg. An experiment incorporating the latter level has recently been completed; a low incidence of hepatoma was found (Wogan *et al.,* 1974). This judgment is also based on a significant incidence of hepatoma in rainbow trout (Halver, 1967) on a diet containing aflatoxin B_1 at 0.4 μg/kg. However, the conclusions based on these data cannot be applied in many food economies, because the alternative is starvation. What other information is available that might shed light on the risk to man?

COMPARATIVE METABOLISM

In vivo metabolism studies with rats (Wogan *et al.,* 1967) and monkeys (Dalezios, 1971) show that aflatoxin B_1 administered intraperitoneally disappears rapidly and that most of the conversion occurs in the liver (Wogan *et al.,* 1967). *In vitro* studies with liver slices and liver cell components have demonstrated that much of the activity is in the microsomal fraction (Portman *et al.,* 1968; Patterson and Allcroft, 1970a,b) but significant activity has also been found in nonmicrosomal preparations (Friedman *et al.,* 1972). More important, *in vitro* studies with livers of species susceptible and resistant to aflatoxin intoxication have shown differences in metabolic rates and patterns (Portman *et al.,* 1968, 1970; Patterson and Allcroft, 1970a,b; Patterson and Roberts, 1970). One such study utilized the observation that phenobarbital-inducible liver microsomal enzymes could convert aflatoxin B_1 to a compound lethal to a special strain of *Salmonella typhimurium* (Garner *et al.,* 1972). These observations open up the possibility of comparing man with appropriate test animals, using human liver specimens made available

through surgical necessity. The last cited reference included one such specimen. More work along these lines is contemplated. Conclusions from such studies must be tempered by judgments derived from other sources, e.g., primate studies, acute and subacute human incidents, and epidemiological studies.

PRIMATE STUDIES

A limited amount of work with primates, involving Macaque (rhesus and cynomolgus) and African monkeys, has been published. The aflatoxin excretion pattern of the rhesus monkey is much different from that of the rat (Shantha *et al.*, 1970), the most intensively studied test animal; moreover, differences in aflatoxin-induced hepatic lesions between the rhesus and African monkey (Alpert *et al.*, 1970; Shank *et al.*, 1971b) suggest that this observation should not be extrapolated to other primate species. Lesions resembling those sometimes seen in man could be induced with aflatoxin in cynomolgus (Shank *et al.*, 1971b) and African monkeys (Alpert *et al.*, 1970), but until recently neither cancerous nor precancerous observations had been made, even with cynomolgus monkeys on a near-lethal dose for 3 years (Cuthbertson *et al.*, 1967). Two reports (Gopalan *et al.*, 1972; Adamson *et al.*, 1973) of aflatoxin-induced hepatic carcinoma in monkeys, involving longer time periods, have now been published.

In the Gopalan study, 2 rhesus monkeys (1 male, 1 female) had been dosed with partially purified aflatoxins (44% B_1, 44% G_1). The initial weight of the male was 1.7 kg and that of the female 2.0 kg. The aflatoxins were administered for the first year by intramuscular injection, 5 days each week, at a rate of 50 μg/animal for the first month and 100 μg/animal for the subsequent 11 months. For the next 4.5 years the male was given a daily oral dose of 200 μg and the female 100 μg, followed by 2.5 more years on an aflatoxin-free diet. At the end of this time the male monkey developed abdominal swelling, generalized edema, and severe jaundice, and was sacrificed after becoming comatose and moribund. Necropsy revealed a massive hepatocellular carcinoma of the giant-cell type. A laparotomy performed at the same period on the female revealed a normal liver by both gross and histological observation.

The Adamson study involved a mixed sex and species group of 40 rhesus and cynomolgus monkeys. One female rhesus, started on aflatoxin B_1 soon after birth, received 50 μg, 3 times every fortnight, as part of its infant feed formula. After 5 months the dose was increased to 200 μg/kg body weight, 5 times every fortnight, as part of a vitamin sandwich. By the time the animal had reached 44 months of age the dose had been increased by several increments to 800 μg/kg body weight, 3 times every fortnight, and held at this level for another 30 months. Since the vitamin sandwich was not completely consumed, the estimated intake of aflatoxin B_1 was about 500 mg of the 840 mg offered during the course of the experiment. For better control of the dose, administration was changed to 800 μg/kg body weight, once weekly by oral intubation. Within hours following the initial dose in this form, the animal became progressively jaundiced and inactive, and was sacrificed after 7 days when found lying in its cage. On necropsy, primary liver carcinoma was observed.

A male rhesus, on a similar regimen for 5 years from birth, died after 4 oral intubations of 400 μg/kg within 2 weeks. Histological observation of the liver revealed small nodules similar to preneoplastic lesions seen in monkeys given *N*-nitrosodiethylamine. The study is continuing with a lower dose level by oral intubation (Dalgard, 1972).

Since rhesus monkeys can normally tolerate an aflatoxin B_1 dose of 800 μg/kg, the acute effects observed are explained on the basis that the chronic administration had impaired the liver to the point that it was now sensitive to the suddenly higher single dose (Dalgard, 1972). This situation would correspond to the adult human case (Bosenberg, 1972) described in the next section of this paper.

The meager primate data have been collated (Table I). From this tabulation an estimate can be made that within the time frame of these studies a daily dose causing serious liver damage in a short period of time is about 0.05 mg/kg body weight and a single dose resulting in death is about 2.2 mg/kg body weight; a daily dose without apparent effect is about 0.01 mg/kg body weight and a single dose without apparent effect is about 1.5 mg/kg body weight. The "apparent" is introduced because the time period and pathological observation were not adequate for a "no effect" conclusion. Administration of about half the acute daily dose for 6 years (about one-third the average life span) can result in liver malignancy.

The difference between "no apparent effect" and liver damage levels is much smaller than would have been anticipated from a classical experiment involving related animals and protocol. These figures are derived from experiments in which a high degree of animal response variability was reported within and between tests conducted by different investigators. The variability is possibly due partly to the different modes of administration, to the sensitivity of the aflatoxin metabolizing enzyme system to environmental influence, and to the different effects of dietary protein composition on animal susceptibility to aflatoxicosis as reported by two investigators (Madhaven and Gopalan, 1965; Deo *et al.*, 1970). The liver injury data in particular are derived from two separate experiments, with one animal on the subacute level in each experiment. With these shortcomings in mind, the data may possibly be used in conjunction with the human incidents to be described to obtain a general idea of the risk to man from various levels of aflatoxin ingestion.

From Recommended Daily Allowance (RDA) figures (Food and Nutrition Board, 1968), the maximum post-weaning food intake per unit of body weight is for children 1–3 years old. From the same charts a 13-kg, 2-year-old child should have an intake of 1175 kcal. Translating this to a meal comprised solely of rice, an occasionally contaminated staple in Thailand (Shank *et al.*, 1972b) and in India (Sreenivasamurthy, 1972), this consumption would be about 380 g of raw rice. Raw rice on the market is normally free of aflatoxin (Shank *et al.*, 1972d). Aflatoxin contamination of prepared rice is sometimes encountered, although contamination levels in the parts per million range (raw rice basis) are not common. Using the aflatoxin B_1 dose figures for primates from the preceding paragraph, the contamination levels would be 1.7 mg/kg (ppm) for a daily dose causing serious liver damage in a short period of time, 75 mg/kg for a single dose resulting in death, 0.34 mg/kg for a daily dose without apparent effect, and 51.3 mg/kg for a single dose without apparent effect. Lest these translations be misinterpreted as recommendations, we wish to stress here the complexity of the problem of making judgments in regard to man and reiterate the need expressed earlier to use all possible approaches: comparative metabolism, primate studies, acute and subacute human incidents, and epidemiological studies.

HUMAN INCIDENTS

Two fatalities involving children, in which aflatoxin was the suspected agent, have been reported. In both cases, samples of the main food consumed prior to the fatal illness were obtained and analyzed. In one case (Bourgeois *et al.*, 1971) a 10-kg child consumed contaminated food for 2 days. We estimate from RDA figures and food composition tables (Watt *et al.*, 1963) that an intake of 1000 g/day of steamed rice contaminated with aflatoxin B_1 at 6

Table I. Summary of Primate Studies; Effect of Aflatoxin B_1 Administered *Per Os*

Monkey			Aflatoxin B_1 dose,[a] mg/kg body wt					
Type	Sex	Diet	Lethal or acute[b,c]	Liver injury	Hepatoma	No effect	Dose form	Reference
Macaque (rhesus)	M	Routine	0.7 daily (6) (1 month)	0.07 daily (1) (6 months)			Mixed cryst. in H_2O suspension, by gavage	Tulpule *et al.*, 1964 Madhavan *et al.*, 1965b
Macaque (rhesus)	M + F	High protein	0.4 daily (4) (20 days)			0.08 daily (2) (46 days)	Mixed cryst. in H_2O suspension, by gavage	Madhavan *et al.*, 1965a
		Low protein	0.08 daily (2) (1 month)					
Macaque (rhesus)	F	Routine	3.0 single (1) (4 days)				Cryst. B_1 in EtOH or EtOH–propylene glycol by gavage	Svoboda *et al.*, 1966
Macaque (rhesus)	M	High protein	0.13 2× weekly (8) (1–19 weeks)				Crude aflatoxins in peanut oil, by gavage	Deo *et al.*, 1970
		Low protein	0.13 2× weekly (16) (1–19 weeks)					
		Routine				0.03 weekly (5) (16 weeks)		
Macaque (rhesus)	M + F	Routine			Est.[d] 0.02 daily (1 M) for 5.5 yr (7.5 yr)	Est. 0.01 daily (1 F) for 5.5 years (7.5 years)	Mixed cryst. in H_2O suspension, by gavage	Gopalan *et al.*, 1972
Macaque (rhesus and cynomolgus)	M + F	Routine			Est. 0.06 daily[e] (1 M, 1 F) (5.8 yr)	Est. 0.023 daily[e] (38 M + F) (6.5 years)	Cryst. B_1 in Me_2SO by vitamin sandwich	Adamson *et al.*, 1973 Dalgard, 1972
Macaque (cynomolgus)	M + F	Routine	0.05 daily (7) (1–3 months)			0.01 daily (4) (3 years)	Contaminated peanut meal in diet	Cuthbertson *et al.*, 1967
Macaque (cynomolgus)	M	Routine	LD_{50} 2.2 single (5) (4 days)				Cryst. B_1 in Me_2SO, by gavage	Rao and Gehring, 1971
Macaque (cynomolgus)	F	Routine	LD_{50} 7.8 single (8) (6 days)			1.5 single (4) (7 days)	Cryst. B_1 with sucrose in gelatin capsule	Shank *et al.*, 1971b
African	M	Routine	0.6 daily (1) (6 days)	0.007 daily (1) (22 days)			Mixed cryst. in corn oil, by gavage	Alpert *et al.*, 1970

[a] Calculated on the basis that aflatoxin G_1 has one-third the potency of B_1 (Butler *et al.*, 1969). [b] Number in parentheses = number of animals. [c] Time period is time to death or duration of experiment. [d] Based on animal weight of 6 kg at 3 yr and 8.5 kg at 5 yr (Krishnamurthi, 1972); introduced intramuscularly for the first year. [e] Distributed over a regimen varying from two to five times fortnightly.

mg/kg (Shank, 1972) resulted in a daily dose of 0.6 mg/kg body weight. In the other case (Serck-Hanssen, 1970) a 36-kg child consumed, for a short period of time, cooked cassava contaminated at 1.7 mg/kg. Calculating from RDA figures and food composition tables (WuLeung *et al.*, 1968), the cooked cassava consumption could be estimated at 2000 g/day and thus a daily aflatoxin B_1 intake of 0.10 mg/kg body weight. In both cases, no other members of either family consuming the same food exhibited any signs of toxicosis. Again calculating from RDA figures, the adult male daily consumption would have been approximately 0.2 mg/kg body weight from the rice or 0.05 mg/kg body weight from the cassava. These dose differences are inadequate to explain the resistance of the adult family members except, perhaps, on the basis of an age-related difference in metabolism.

Evidence of sublethal effects of aflatoxins comes from instances in which aflatoxin-contaminated peanut meals were inadvertently used as a source of protein for kwashiorkor therapy. In one situation in Senegal (Payet *et al.*, 1966), infants less than 1 year old each received 70–140 g of peanut meal per day for 10 months. The meal samples were later found to be contaminated with aflatoxin (types unspecified) at 0.5–1.0 mg/kg, providing an aflatoxin intake range of 35–140 μg/day. We estimate that this represents a daily intake of 5–20 μg/kg body weight. Two of the children were located for liver biopsies 4 and 6 years after consumption of the contaminated meal. One child had gross abnormalities in the liver structure, persisting through the sixth year; the other child had minor liver abnormalities at 4 years and recovered to completely normal at 6 years. There is no record of other challenges the children may have experienced in the intervening years.

A more extensive experience was recorded for children under treatment for kwashiorkor in Mysore, India (Amla *et al.*, 1970, 1971). Twenty children, including one normal child treated for nephritis, ranging in age from 1.5 to 5 years, were given daily dietary supplements of 30–60 g of peanut meal, later found to contain aflatoxin B_1 at 0.3 mg/kg. We estimate that the daily aflatoxin intake was thus 9–18 μg or an average of 1.1 μg/kg body weight. The contaminated meal had been consumed for periods ranging from 5 days to 1 month. Three of the children on the contaminated meal supplement for 17 days displayed all the signs of liver cirrhosis, the observation that led to the analysis of the peanut meal. Clinical followup, including palpation and liver biopsies, when allowed, showed a general picture of gradual transition from fatty liver to cirrhotic liver over a 1-year period following withdrawal of the toxic meal; characteristic clinical and histopathological lesions were not evident before 6 months. The histopathological changes correlated with the duration of toxic meal ingestion. Observations of the eating habits of the families from which the children came indicated that foodstuffs likely to be contaminated with aflatoxins were not a usual part of their diet. In no case was cirrhosis noted in liver biopsy material nor were clinical symptoms observed when peanut meals with an aflatoxin content averaging 15 μg/kg (Sreenivasamurthy, 1973) had been used for kwashiorkor therapy.

On the basis of these meager data one could surmise that there is an age period of maximum risk to aflatoxin intoxication in the very early years that may coincide with the 1–3-year age group in India most vulnerable to childhood cirrhosis (Amla *et al.*, 1970; Robinson, 1967). The general level at which an effect was observed (Payet, 5–20 μg/kg body weight; Amla *et al.*, 0.7–1.5 μg/kg body weight) in children suffering from kwashiorkor is similar to that causing "apparent effect" in nonhuman primates (10 μg/kg body weight). There is no evidence in this human experience to resolve the conflicting results of the primate studies (Madhavan *et al.*, 1965a,b; Deo *et al.*, 1970) with regard to the effect of dietary protein level on susceptibility to aflatoxin intoxication. Childhood cirrhosis in India seems to correlate with religious dietary patterns (Amla *et al.*, 1970) and more than 90% of the cases in the Mysore Cheluvamba hospital are reported to have come from the middle income group. Dietary items common to the cirrhotic patients were parboiled rice and crude peanut oil. Analysis of market samples showed occasional aflatoxin contamination of rice and almost universal contamination of the crude peanut oil, with levels of aflatoxin B_1 ranging to 0.1 mg/kg. Mothers' breast milk was also found to contain aflatoxin B_1, a finding corroborated by a similar study in Bangalore, India (Robinson, 1967).

Aflatoxin B_1 was found in the urine of both normal and cirrhotic children (cirrhotic, 15/250 = 6%; normal, 7/74 = 11%), indicating that exposure was recent but not its degree. Amounts found in 24-hr urine collections ranged from 0.02 to 0.05 μg. No aflatoxin M_1 was observed. In a study in the Philippines (Campbell *et al.*, 1970), however, where childhood cirrhosis is not reported as endemic, only aflatoxin M_1 was excreted in the urine and secreted in mothers' milk. In this study a correlation between ingestion and excretion was possible. Aflatoxin M_1 was observable in the urine (test sensitivity about 5 μg/l.) when total ingestion levels of aflatoxin B_1 exceeded 15 μg/day (0.8–1.0 μg/kg body weight). Contaminated peanut butter was the aflatoxin source in all cases. Analyses of market samples had shown that locally manufactured peanut butter was almost always contaminated with aflatoxins, with a median near 0.5 mg/kg. This comparison of the Indian and Philippine studies indicates possible differences in responses to similar levels of exposure to aflatoxin, which may be related to the apparent difference in the way each population metabolizes aflatoxin (B_1 *vs.* M_1 in the urine). Because of the importance of the conclusion that may be drawn from these comparisons, the validity of the data, particularly the lack of reported childhood cirrhosis in the Philippines and the presence of aflatoxin B_1 in the urine and milk of the Indian population, requires confirmation by collaboration of the respective investigators.

A different manifestation of possible aflatoxin toxicosis was found in Thailand (Shank *et al.*, 1971a). Reports indicate that several hundred children in Thailand between the ages of 1 and 13 years may die annually of a disease involving acute encephalopathy and fatty degeneration of the viscera (EFDV), similar in detail to Reye's syndrome (Bourgeois *et al.*, 1971). The extent of aflatoxin contamination in food samples from Thai markets seems to parallel the incidence of this acute disease, on both seasonal and geographical bases. Aflatoxin assays of autopsy specimens from 23 children who had died of EFDV revealed aflatoxin B_1 in one or more of the specimens from 22 of the children. Levels were as high as 0.093 mg/kg in a liver, 0.123 mg/kg in a stool, 0.127 mg/kg in a specimen of stomach and intestinal contents, and 8 mg/l. in a specimen of bile; traces of aflatoxin B_1 were found in brain, kidney, and urine. In some specimens of liver, stool, and gut contents, aflatoxin B_2 was also found. Urine samples from another 51 EFDV cases were analyzed for aflatoxins; aflatoxin B_1 was found in eight of the specimens, aflatoxin M_1 in two of the eight. Traces of aflatoxin B_1 were found in tissues from 11 of 15 children dead from causes other than EFDV, indicating some degree of prior exposure; no aflatoxins were found in urine specimens from 39 healthy children 1–5 years of age, indicating no recent exposure to aflatoxin. A pathology closely resembling EFDV in children was found in cynomolgus monkeys that had received a single dose of aflatoxin B_1 at 13.5 mg/kg body weight (Bourgeois *et al.*, 1971). The residues of aflatoxin found in the monkey tissues were also similar to the human cases; aflatoxin could be found in the brain, liver, kidney, heart,

Table II. Comparison of Primary Hepatoma Incidence (Crude Rate) and Exposure to Aflatoxins in Various Populations

Region	Aflatoxin exposure		Hepatoma incidence (cases/10^5 per yr) Male	Female	Total	Reference
	Contaminated peanuts					
Swaziland	No. examined	% positive				
Highlands	37	8			2.2	Keen and Martin, 1971a,b
Middlelands	67	25			4.0	
Lowlands	26	54			9.7	
Total population			8.6	1.6		
	Family meals					
	Av B_1,	No.	%			
Kenya (Muranga)	μg/kg	examined	positive			
Highlands	2.9	808	4.2	3.1	0	Peers and Linsell, 1973
Middlelands	3.2	808	6.5	10.8	3.3	
Lowlands	3.7	816	9.6	12.1	5.4	
Total population				10.5	3.8	
	Foodstuffs					
	Av B_1,	No.	%			
Uganda	μg/kg	examined	positive			
Karamajang	189	105	36		3.5	Alpert *et al.*, 1968, 1971, 1972[a]
Buganda	39	149	11		2.0	
West Nile, Achuli, Soga, and Ankoli	44	128	8		2.3	
	Av daily intake from family meal of aflatoxin B_1, ng/kg body wt					
Thailand	Peak season	Yearly mean				
Singburi area	118–126	51–55			14.0	Shank *et al.*, 1972a,b
Ratburi area	73–108	31–48			7.6	
Songkhla area	<1–1	0–<1			2.0	

[a] Data on aflatoxin contamination recalculated from data cards on hepatoma incidence in Karamajang corrected on the basis of Alpert *et al.*, 1971.

blood, and bile of some animals 148 hr after toxin administration (Shank *et al.*, 1971b).

The evidence for acute aflatoxicosis in humans has so far involved children, but adults with predisposing liver ailments might also be in jeopardy. One case history (Bosenberg, 1972) concerned a man suffering from a disease related to the storage of iron, which had produced a liver sclerosis not yet progressed to the point that it could explain his sudden death. An alert pathologist submitted the *post mortem* liver for aflatoxin analysis. A positive finding of aflatoxin B_1 in the liver plus the information supplied by the man's wife that he had been eating unusually large quantities of various nuts just before becoming acutely ill, support the possibility that aflatoxicosis may have been involved. Although no aflatoxin was found in the remaining nuts, we think that the ingestion of high levels of aflatoxin from a few contaminated kernels is very possible, based on our experience with the distribution of aflatoxin in nut meats.

EPIDEMIOLOGY

Epidemiological studies have concentrated on a possible correlation between aflatoxin ingestion and primary liver cancer. Interpretation of all such studies suffers from a number of complicating factors: (1) an unknown, probably long and variable induction period requires that current exposure to aflatoxin be typical of past exposure; (2) average exposure from commodity surveys does not reflect the distribution and level of individual exposure, particularly if the populations under study are not homogeneous; (3) cancer identification, registration, and census data are usually poor in areas of highest hepatoma incidence; and (4) possible unknown vectors of hepatoma such as other mycotoxins, or effect of diet as a predisposing factor, are not considered.

In the studies reported, the first problem is minimized by using indigenous rural populations with rather stable and simple eating habits. In two of the studies (Peers and Linsell, 1973; Shank *et al.*, 1972b), the incidence and individual level of exposure are estimated from analyses of typical family meals and in three of the studies (Peers and Linsell, 1973; Alpert *et al.*, 1971; Shank *et al.*, 1972b) variations in eating habits are minimized by studying small homogeneous populations. In three of the four completed studies (Keen and Martin, 1971b; Peers and Linsell, 1973; Alpert *et al.*, 1971) the epidemiological data were obtained from contiguous areas with similar health services and reporting facilities; in the fourth, the study group developed their own epidemiological data in cooperation with local hospitals. In one study (Purchase and Goncalves, 1971), not yet completed, the foods were examined for sterigmatocystin as well as aflatoxin; aflatoxin was found but not sterigmatocystin. With the unknown quantity of other vectors, the best basis for establishing a correlation between the two factors is the consistency of the correlation in a number of independent studies of populations with different exposures and food habits.

All four completed studies so far reported (Keen and

Table III. Comparison of Sex and Age Frequency Distribution of Hepatoma in Different Regions

Region	Period	Male/female	Peak ages, yr	Max frequency: Ages, yr	Max frequency: % of all cases	Reference
Swaziland		5.4				Keen and Martin, 1971b
Uganda	1964–1966	3.3	35–45	25–55	75	Alpert *et al.*, 1968
Kenya	1967–1970	2.8				Peers and Linsell, 1973
Mozambique	1968–1970	2.2	30–39	20–49	76	Purchase and Goncalves, 1971
Thailand	1962–1968	6		30–59	88	Shank *et al.*, 1972a
Bangkok and Chiang Mai	1963–1969	2.5	40–60	30–60	77	Shank *et al.*, 1972c
Ratburi and Songkhla	1969–1970	5		36–65	75	Shank *et al.*, 1972a
United States	1969					
	All races	2.0	55–75	55+	78	Biometry Branch, 1971
	Caucasian	1.8				
	Negro	3.2				

Table IV. Aflatoxin Contamination of Prepared Foods and Household Rice in Selected Areas of Thailand (Shank *et al.*, 1972b)

Area	Prepared foods: Aflatoxin contam., %	Prepared foods: Highest single ingestion of aflatoxins, μg/kg body wt per day	Rice: Aflatoxin contam., % of sample	Rice: Highest single aflatoxins level, μg/kg	Hepatoma incidence, cases/10^5 per yr
Singburi	2	13	3	600	14.0
Ratburi	5	3.2	10	180	7.6
Songkhla	0.6	1.1	1	71	2.0

Martin, 1971b; Alpert *et al.*, 1968; Peers and Linsell, 1973; Shank *et al.*, 1972a) show a positive correlation between aflatoxin ingestion and incidence of primary hepatoma (Table II). Possible evidence that the same cause-effect relationships (aflatoxin ingestion-hepatoma incidence) are being observed is the relative consistency among these separate studies of the differentials in sex susceptibility and the age frequency of occurrence (Table III). Two of the reported studies (Alpert *et al.*, 1968; Shank *et al.*, 1972a) correlate in both respects. In two of the studies (Keen and Martin, 1971b; Peers and Linsell, 1973) only the sex susceptibility could be derived from the data presented and both show a high male/female incidence similar to the first two. The report of a study in progress (Purchase and Goncalves, 1971) contains age frequency data that appear consistent with the others. This leaves an unanswered question. Does a smaller differential in sex susceptibility and greater age for peak frequency of hepatoma as seen (Table III) in the United States (Biometry Branch, 1971) or the smaller differential in sex susceptibility as seen in South Africa (Purchase and Goncalves, 1971) mean that a different phenomenon is being observed, or is this a manifestation of lower dose levels, or differences in genetic susceptibility?

Other observations help bolster the conclusion of a cause-effect relationship between aflatoxin ingestion and incidence of primary hepatoma in some of the areas. In both Swaziland (Keen and Martin, 1971a) and Kenya (Peers and Linsell, 1973) the climate ranges from temperate to tropical as one goes from the high to the lowlands, the same direction that favors the competitive position of *Aspergillus flavus*. In Swaziland (Keen and Martin, 1971b) the incidence of primary hepatoma correlates with both the consumption level and aflatoxin level of peanuts, and the general quality of agronomic practices. A correlation has also been found between the incidence of hepatoma in two comingled tribes (Swazi *vs.* Shangaan) and the predilection of the Shangaan tribe for consuming peanuts. In Thailand (Shank *et al.*, 1972b) the incidence of primary hepatoma (Table II) correlates with the incidence of aflatoxin-contaminated prepared foods and the aflatoxin level in individual meals as well as with the incidence and level of aflatoxins in the dietary staple, rice, as sampled from the individual homes (Table IV). Aflatoxins from peanuts, which are eaten outside of the regular meals, are not included in this observation of foods consumed.

This last observation opens up a new approach to an assessment of the aflatoxin hazard. It was observed that the level of contamination of peanuts was the same in all three Thai areas studied, and that 10-12-kg children were seen eating up to 250 g of peanuts in 1 day. The exposure to aflatoxin by this route must be considerable when one considers a 54% incidence of aflatoxin-contaminated peanuts, with aflatoxin B_1 levels averaging 0.87 mg/kg for the contaminated nuts and occasionally as high as 6.5 mg/kg (Shank *et al.*, 1972b). Yet the exposure to aflatoxins in the household meals, superimposed on this background, correlated with a difference in the hepatoma rates for the three areas, and the lowest hepatoma rate for a Thai area studied is on a par with those from low rate areas (Table IV). Although oversimplified, this may suggest the possibility of natural selection of populations in areas of high

Table V. Aflatoxin B_1 Contamination of Selected Foods

Country	Commodity	No. samples, contam./examined	Av aflatoxin B_1 in positive samples, μg/kg	Reference
South Africa	Peanuts	5/67	2 samples > 1000	Purchase and Goncalves, 1971
	Corn	2/52		
	Rice	1/23	>1000	
	Manioc	1/8	>1000	
Uganda	Beans	15/64	500	Alpert *et al.*, 1971; Alpert, 1972
	Peanuts	29/150	363	
	Corn	19/48	133	
	Sorghum	16/69	152	
	Cassava	2/34	879	
	Millet	6/55	26	
	Peas	2/19	30	
	Sin-sin	2/11	33	
	Food mixture	2/15	73	
	Rice	0/11	0	
Thailand	Peanuts	116/216	872	Shank *et al.*, 1972d
	Corn	22/62	265	
	Chili pepper	12/106	80	
	Millet	5/44	38	
	Dried fish/shrimp	7/139	104	
	Mung beans	7/140	~10	
	Beans, general	10/322	106	
	Cassava	2/65	60	
	Rice	7/364	~10	
Philippines	Peanuts, whole	80/100	98	Campbell and Salamat, 1971[a]
	Peanut butter	145/149	213	
	Peanut candies	47/60	38	
	Corn, whole	95/98	110	
	Corn products	22/32	32	
	Rice products	1/72	<1	
	Root and tuber products	48/62	44	
	Beans	26/29	35	
	Commercial livestock feeds	39/42	28	

[a] Additional unpublished data tabulated.

contamination to increase the general resistance. This possibility would be better supported if complete disease incidence data were available.

The epidemiological studies also provide a picture of the relative susceptibility of various commodities to aflatoxin contamination. In Swaziland (Keen and Martin, 1971b) the only commodity examined was peanuts. In Kenya (Peers and Linsell, 1973) the commodities most frequently found in aflatoxin-positive diets were corn, millet, sorghum, pigeon peas, and yams. More detailed information from the other four areas is presented in Table V. Peanuts and corn appear to be highly susceptible commodities in all four areas. Rice appears as a relatively low risk item, yet examination of prepared rice for family meals in Thailand revealed considerable contamination (Shank *et al.*, 1972c). Rice harvesting practices apparently provide little opportunity for mold growth, but poor storage practices for both raw and cooked rice at the individual household level, as observed in Thailand and India (Sreenivasamurthy, 1972), can result in considerable contamination. This observation should also alert future investigators to the possible fallacy of extrapolating from market samples to the cookpot, although the report from Uganda (Alpert *et al.*, 1971) notes that the source of food, whether from cookpot, village market, or home grown, was not significant in regard to level or incidence of aflatoxin contamination.

Interpretation of the epidemiological data brings up the question of genetic differences in susceptibility. The different manifestations of aflatoxin poisoning as seen in India, Indonesia, and Africa cause one to wonder: are the differences genetic or merely observational? There is a general feeling from observations of migrant groups (Stewart, 1965) that genetics has very little, if anything, to do with incidence of primary hepatoma.

The word aflatoxin has almost become synonymous with hepatoma and most of the epidemiological effort appears to be concentrated on this manifestation of aflatoxin poisoning. To retain perspective, evidence must be considered that the kidney may be the target organ in the guinea pig (Madhavan and Rao, 1967), in the hamster (Herrold, 1971), and sometimes in the rat (Butler and Lijinsky, 1971), particularly when on a low lipotrope diet (Newberne *et al.*, 1968); the colon may be the target organ of a rat deprived of vitamin A (Newberne and Rogers, 1972) or the lung the target organ of a mouse (Wieder *et al.*, 1968). Moreover, aflatoxins have been suggested as a factor in encephalopathy in a human epidemic situation

(Bourgeois *et al.*, 1971; Shank *et al.*, 1971a). The report (Grice *et al.*, 1973) should also be considered that fetal or suckling rat exposure to aflatoxin or its metabolites through the placenta and/or the milk results in benign and malignant tumors and tumor-like lesions of the liver.

Although this paper deals mainly with the aflatoxins, other mycotoxins have been receiving their share of attention. From mold-related episodes in the veterinary literature, from research on antibiotics that proved more toxic to man than to microbe, from toxicological screening of mold collections, and from pure research into the metabolic products of molds, lists of potentially significant mycotoxins have been developed (Ajl *et al.*, 1971). From considerations of the potential effects of ingestion of subacute levels and the prevalence of the producing molds, we feel that some attention should now be focused on sterigmatocystin (produced by *Aspergillus versicolor, inter alia*), patulin (produced by *Penicillium expansum, P. urticae, inter alia*), penicillic acid (produced by *Penicillium cyclopium, P. palitans, inter alia*), members of the trichothecene group (produced by various *Fusaria*, particularly *F. tricinctum*), luteoskyrin (produced by *Penicillium islandicum*), and citrinin (produced by *Penicillium citrinum, P. viridicatum, inter alia*). Two of these mycotoxins have been shown to have carcinogenic potential on ingestion: sterigmatocystin (Purchase and van der Watt, 1970) and luteoskyrin (Uraguchi *et al.*, 1972).

LITERATURE CITED

Adamson, R. H., Correa, P., Dalgard, D. W., *J. Nat. Cancer Inst.* **50,** 549 (1973).

Ajl, S. J., Ciegler, A., Kadis, S., Montie, T. C., Weinbaum, G., *Microbial Toxins* **6–7** (1971); **8** (1972).

Alpert, M. E., personal communication, 1972.

Alpert, M. E., Hutt, M. S. R., Davidson, C. S., *Lancet,* 1265 (1968).

Alpert, M. E., Hutt, M. S. R., Wogan, G. N., Davidson, C. S., *Cancer* **28,** 253 (1971).

Alpert, M. E., Serck-Hanssen, A., Rajagopolan, B., *Arch. Environ. Health* **20,** 723 (1970).

Amla, I., Kamala, C. S., Gopalakrishna, G. S., Jayaraj, A. P., Sreenivasamurthy, V., Parpia, H. A. B., *Amer. J. Clin. Nutr.* **24,** 609 (1971).

Amla, I., Shyamala, K., Sreenivasamurthy, V., Jayaraj, A. P., Parpia, H. A. B., *Indian Pediat.* **7,** 262 (1970).

Armbrecht, B. H., Wiseman, H. C., Shalkop, W. T., Geleta, J. H., *Environ. Physiol.* **1,** 198 (1971).

Bamburg, J. R., Strong, F. M., *Microbial Toxins* **7,** 207 (1971).

Barger, G., "Ergot and Ergotism," Gurney & Jackson, London, 1931, p 85.

Biometry Branch, National Cancer Institute, Preliminary Report, Third National Cancer Survey, 1969 Incidence, U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, DHEW Publication No. (NIH) 72-128, Sept 9, 1971.

Bosenberg, H., *Zbl. Bakt. Hyg., I. Abt. Orig. A* **220,** 252 (1972).

Bourgeois, C. H., Shank, R. C., Grossman, R. A., Johnsen, D. O., Wooding, W. L., Chandavimol, P., *Lab. Invest.* **24,** 206 (1971).

Butler, W. H., Greenblatt, M., Lijinsky, W., *Cancer Res.* **29,** 2206 (1969).

Butler, W. H., Lijinksy, W., *J. Pathol.* **102,** 209 (1971).

Campbell, T. C., Caedo, J. P., Bulatao-Jayme, J., Salamat, L., Engel, R. W., *Nature (London)* **227,** 403 (1970).

Campbell, T. C., Salamat, L., in "Mycotoxins in Human Health," Purchase, I. F. H., Ed., MacMillan Press, London, 1971, pp 263–269.

Cuthbertson, W. F. J., Laursen, A. C., Pratt, D. A. H., *Brit. J. Nutr.* **21,** 893 (1967).

Dalezios, J. I., "Aflatoxin P_1: A New Metabolite of Aflatoxin B_1, Its Isolation and Identification," Sc.D. Dissertation, Massachusetts Institute of Technology, Jan 1971.

Dalgard, D., Hazleton Laboratories, Falls Church, Va., private communication, December 1972.

Deo, M. C., Dayal, Y., Ramalingaswami, V., *J. Pathol.* **101,** 47 + 4 plates (1970).

Eppley, R. M., Bailey, W. J., *Science* **181,** 758 (1973).

Food and Nutrition Board, National Research Council, National Academy of Sciences, "Recommended Dietary Allowances," 7th ed, Publication 1694, Washington, D. C., 1968.

Forgacs, R., *Microbial Toxins* **8,** 95 (1972).

Friedman, L., Yin, L., Verrett, M. J., *Toxicol. Appl. Pharmacol.* **23,** 385 (1972).

Garner, R. C., Miller, E. C., Miller, J. A., *Cancer Res.* **32,** 2058 (1972).

Gopalan, C., Tulpule, P. G., Krishnamurthi, D., *Food Cosmet. Toxicol.* **10,** 519 (1972).

Grice, H. C., Moodie, C. A., Smith, D. C., *Cancer Res.* **33,** 262 (1973).

Gumbmann, M. R., Williams, S. N., Booth, A. N., Vohra, P., Ernst, R. A., Bethard, M., *Proc. Soc. Exp. Biol. Med.* **134,** 683 (1970).

Halver, J. E., *U. S. Bur. Sport Fish. Wildl., Res. Rep.* **No. 70,** 78 (1967).

Herrold, K. M., *Brit. J. Cancer* **23,** 655 (1971).

Joffe, A. Z., *Microbial Toxins* **7,** 139 (1971).

Keen, P., Martin, P., *Trop. Geogr. Med.* **23,** 35 (1971a).

Keen, P., Martin, P., *Trop. Geogr. Med.* **23,** 44 (1971b).

Krishnamurthi, D., personal communication, Oct 1972.

Krushchev, N., "Khrushchev Remembers," Little, Brown, and Co., Boston, Mass., 1970, pp 110–114.

Lancaster, M. C., Jenkins, F. P., Philp, J. Mcl. (Part 1); Sargeant, K., Sheridan, A., O'Kelly, J., Carnaghan, R. B. A. (Part 2), *Nature (London)* **192,** 1095 (1961).

Madhavan, T. V., Gopalan, C., *Arch. Pathol.* **80,** 123 (1965).

Madhavan, T. V., Rao, K., *J. Pathol. Bacteriol.* **93,** 329 (1967).

Madhavan, T. V., Rao, K. S., Tulpule, P. G., *Indian J. Med. Res.* **53,** 984 (1965a).

Madhavan, T. V., Tulpule, P. C., Gopalan, C., *Arch. Pathol.* **79,** 466 (1965b).

Mirocha, C. J., Pathre, S., *Appl. Microbiol.* **26,** 719 (1973).

Newberne, P. M., Rogers, A. E., *Toxicol. Appl. Pharmacol.* **22,** 280 (1972).

Newberne, P. M., Rogers, A. E., Wogan, G. N., *J. Nutr.* **94,** 331 (1968).

Patterson, D. S. P., Allcroft, R., *Food Cosmet. Toxicol.* **8,** 45 (1970a).

Patterson, D. S. P., Allcroft, R., *J. S. Afr. Chem. Inst.* **22,** 556 (1970b).

Patterson, D. S. P., Roberts, B. A., *Food Cosmet. Toxicol.* **8,** 527 (1970).

Payet, M., Cros, J., Guenum, C., Sankale, M., Monlanier, M., *Presse Med.* **74,** 649 (1966).

Peers, F. G., Linsell, C. A., *Brit. J. Cancer* **27,** 473 (1973).

Portman, R. S., Plowman, K. M., Campbell, T. C., *Biochem. Biophys. Res. Commun.* **33,** 711 (1968).

Portman, R. S., Plowman, K. M., Campbell, T. C., *Biochim. Biophys. Acta* **208,** 487 (1970).

Protein Advisory Group, WHO-FAO-UNICEF, *Nutrition Document R. 3*/Add. **23,** March 1962.

Protein Advisory Group, FAO-WHO-UNICEF, Aug 17–19, 1966.

Purchase, I. F. H., Goncalves, T., in "Mycotoxins in Human Health," Purchase, I. F. H., Ed., MacMillan Press, London, 1971, pp 263–269.

Purchase, I. F. H., van der Watt, J. J., *Food Cosmet. Toxicol.* **8,** 289 (1970).

Rao, K. S., Gehring, P. J., *Toxicol. Appl. Pharmacol.* **19,** 169 (1971).

Robinson, P., *Clin. Pediat.* **6,** 57 (1967).

Rogers, A. E., Newberne, P. M., *Cancer Res.* **29,** 1965 (1969).

Rogers, A. E., Newberne, P. M., *Toxicol. Appl. Pharmacol.* **20,** 113 (1971).

Saito, M., Enomoto, M., Tatsuno, T., *Microbial Toxins* **6,** 299 (1971).

Serck-Hanssen, A., *Arch. Environ. Health* **20,** 729 (1970).

Shank, R. C., private communication, July 1972.

Shank, R. C., Bhamarapravati, M., Gordon. J. E., Wogan, G. N., *Food Cosmet. Toxicol.* **10,** 171 (1972a).

Shank, R. C., Bourgeois, C. H., Keschamras, N., Chandavimol, P., *Food Cosmet. Toxicol.* **9,** 501 (1971a).

Shank, R. C., Gordon, J. E., Wogan, G. N., Nondasuta, A., Subhawani, B., *Food Cosmet. Toxicol.* **10,** 71 (1972b).

Shank, R. C., Johnsen, D. O., Tanticharoenyos, P., Wooding, W. C., Bourgeois, C. H., *Toxicol. Appl. Pharmacol.* **20,** 227 (1971b).

Shank, R. C., Siddhichai, P., Subhamani, B., Bhamarapravati, M., Gordon, J. E., Wogan, G. N., *Food Cosmet. Toxicol.* **10,** 181 (1972c).

Shank, R. C., Wogan, G. N., Gibson, J. B., Nondasuta, A., *Food Cosmet. Toxicol.* **10,** 61 (1972d).

Shantha, T., Sreenivasamurthy, V., Parpia, H. A. B., *J. Food Sci. Technol. (Mysore)* **7,** 135 (1970).

Sreenivasamurthy, V., personal communication, Dec 1972.

Sreenivasamurthy, V., personal communication, March 1973.

Stewart, H. L., in "Primary Hepatoma," Burdette, W. J., Ed., University of Utah Press, Salt Lake City, Utah, 1965.

Svoboda, D., Grady, H. J., Higginson, J., *Amer. J. Pathol.* **49,** 1023 (1966).

Tulpule, P. G., Madhavan, T. V., Gopalan, C., *Lancet* **1,** 962 (1964).

Uraguchi, K., *Microbial Toxins* **6,** 367 (1971).

Uraguchi, K., Saito, M., Noguchi, Y., Takahashi, K., Enomoto, M., Tatsuno, T., *Food Cosmet. Toxicol.* **10,** 193 (1972).

Watt, B. K., Merrill, A. L., Pecot, R. K., Adams, C. F., Orr, M. L., Miller, D. F., "Composition of Foods," Agriculture Handbook No. 8, U. S. Department of Agriculture, Agricultural Research Service, Revised 1963.
Wieder, R., Wogan, G. N., Shimkin, M. B., *J. Nat. Cancer Inst.* 40, 1195 (1968).
Wogan, G. N., *Fed. Proc., Fed. Amer. Soc. Exp. Biol.* **27**, 932 (1968).
Wogan, G. N., Edwards, G. S., Shank, R. C., *Cancer Res.* **27**, 1729 (1967).
Wogan, G. N., Newberne, P. M., *Cancer Res.* **27**, 2370 (1967).
Wogan, G. N., Paglialunga, S., Newberne, P. M., *Food Cosmet. Toxicol.*, submitted for publication (1974).
WuLeung, W. T., Busson, F., Jardin, C., "Food Composition Table for Use in Africa," U. S. Department of Health, Education, and Welfare, Public Health Service, National Center for Chronic Disease Control, Bethesda, Md., 1968.

Received for review January 15, 1974. Accepted June 28, 1974. A condensed version of this paper was presented at the IXth International Congress of Nutrition, Mexico City, Mexico, Sept 10, 1972, and published in the Proceedings of that Meeting.

Characterization of Two Isomers of 8′-Hydroxyzearalenone and Other Derivatives of Zearalenone

Richard A. Jackson, Stuart W. Fenton, Chester J. Mirocha,* and George Davis

Two naturally occurring derivatives of zearalenone synthesized by *Fusarium roseum* were shown to be isomers of 8′-hydroxyzearalenone. Their structure was proven by comparing their fragmentation pattern (mass spectroscopy) with the parent compound (zearalenone). The two derivatives differed from zearalenone by two unique fragments, *i.e.* *m/e* 95 and 110 *vs.* *m/e* 97 and 112 for the parent compound. The major fragments of the mass spectrum were followed by labeling the aromatic ring with Br. In addition to spectrometric evidence, the reduced form of the parent compound (zearalenone) was synthesized from both isomers. The two isomers of 8′-hydroxyzearalenone, also known as F-5-3 and F-5-4, were not biologically active in the rat uterotropic bioassay for estrogens.

Fusarium roseum (Cke) snyd. & Hans., "Graminearum" (*Gibberella zeae* (Schw.) Petch), is a storage fungus which under certain conditions of moisture and temperature infects maize and produces a mycotoxin called F-2 or zearalenone. When such corn is fed to swine, it causes the estrogenic syndrome which involves primarily the genital system; in the prepuberal gilt, the vulva becomes swollen and edematous and sometimes, in severe cases, it may progress to vaginal prolapse; the uterus is enlarged, edematous, and tortuous, with some atrophy of the ovaries. The young males may undergo a feminizing effect, with atrophy of the testes and enlargement of the mammary glands.

The nature and effects of this mycotoxin have been investigated by Stob *et al.* (1962), Christensen *et al.* (1965), and Mirocha and Christensen (1971).

The structure of zearalenone (I) (Figure 1) was determined by Urry *et al.* (1966) as 6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)resorcylic acid lactone. Mirocha and Christensen (1971) and Mirocha *et al.* (1968) reported seven other naturally occurring derivatives of zearalenone, two of which are described here, namely F-5-3 and F-5-4 (II). Bolliger and Tamm (1972) reported on the structure of II plus two other derivatives, 5-formylzearalenone and 7′-dehydrozearalenone.

Elemental analysis and high-resolution mass spectra established the formula $C_{18}H_{22}O_6$ and a molecular weight of 334 for isomers of II (Figure 2D, E), insoluble in water, benzene, and petroleum ether and slightly soluble in methylene chloride, and ethyl acetate, acetonitrile, acetone, ethyl alcohol, and diethyl ether.

The objectives of this research were to determine the chemical structures of F-5-3 and F-5-4 and study the mass spectroscopy of the zearalenone.

Department of Chemistry (R.A.J., S.W.F.) and Department of Plant Pathology (C.J.M., G.D.), University of Minnesota, Minneapolis and St. Paul, Minnesota 55101.

MATERIALS AND METHODS

Spectrometric Examination. Mass spectra were obtained with an AEI MS-30 double focusing mass spectrometer. Low-resolution spectra were taken at a resolution of 1000 and a scan speed of 10 sec/decade. The source was heated to 200° and the sample probe was heated as required to get a sufficiently high ion current. Unless otherwise specified, the spectra were obtained at 70 eV. High-resolution mass spectra were obtained using an internal perfluorokerosene reference at a resolution of 5000 and a scan speed of 30 sec/decade. Mass spectral data were calculated with an on-line "Digital pdp8" computer. Gc-mass spectral data were obtained on an LKB-9000 gc-mass spectrometer. Gc-mass spectral data as well as low-resolution mass spectra were plotted by hand.

Infrared spectra were obtained on Beckman IR-12, Perkin-Elmer 521, or Perkin-Elmer 257 infrared spectrophotometers. Melting points were obtained with a Fischer Johns melting point apparatus. Analytical tlc was done on unactivated Eastman ±6060 Chromagram silica tlc plates containing a fluorescent indicator. All of the compounds which contained an aromatic moiety appeared either as dark purple spots against an orange fluorescent background when viewed under an ultraviolet light, or as intense blue or green fluorescent spots against an orange fluorescent background.

Biosynthesis and Preparation of Zearalenone Derivatives. The isomers of II are chemically similar to zearalenone and have been isolated from cultures of *F. roseum* "Graminearum," an isolate designated as Mapleton No. 10. Spores of the fungus were seeded onto previously autoclaved rice at a moisture content of 40%, and then incubated at 25° for 2 weeks followed by 4 weeks at 12°. The culture was dried, ground in a Stein Mill, and moistened with water (15% w/v) before extracting with ethyl acetate. The ethyl acetate was concentrated under vacuum, and the residue partitioned between equal volumes of petroleum ether (bp 30–60°) and acetonitrile. The acetonitrile