1392 (32

FAO/UNEP/USSR

International Training Course

«TRAINING ACTIVITIES ON FOOD CONTAMINATION CONTROL AND MONITORING WITH SPECIAL REFERENCE TO MYCOTOXINS»

P. M. NEWBERNE

INTERACTION OF NUTRIENTS AND OTHER FACTORS WITH MYCOTOXINS

Centre of International Projects, GKNT

Moscow, 1985

Interaction of Nutrients and Other Factors with Mycotoxins

Paul M. Newberne

Professor Emeritus/Senior Lecturer

Massachusetts Institute of Technology

Cambridge, MA 02139

and

Professor of Pathology

Boston University School of Medicine

Introduction

Throughout recorded history diseases of man and lower animals have been associated with metabolites of certain molds growing on foodstuffs. However, an understanding of the relationship between the diseases, the molds, the food and its nutrient content has begun to develop only in recent years. It has been known since the turn of the century that certain fungi produce toxic metabolites eliciting biologic responses in both man and animals. As early as the 19th century, a disease associated with the consumption of discolored "yellowed" rice was recognized in Japan and established as a toxicologic entity. This disease has been identified as the "yellowed rice" syndrome. Similarly, alimentry toxic aleukia (ATA), associated with overwintered wheat, affected both man and animals in Russia; this disease has been known for many decades. Centuries prior to these observations, poisoning was often observed in human populations, associated with the ingestion of flour and bread, later

3667

identified as being contaminated with a fungus; this created widespread epidemics of ergotism in Europe - known to the ancients as St. Anthony's fire. Thus, retrospectively, ergot poisoning was recognized as the first of many mycotoxicoses.

Despite the early recognition of ergot poisoning and the toxicoses associated with yellow rice and overwintered grain, the mycotoxicoses remained a generally neglected category of diseases until 1960. A serious outbreak of a toxic disease in poultry in England precipitated scientific investigations which delineated the nature of the disease presently known as aflatoxicosis. This outbreak led to the realization that mycotoxins, and especially aflatoxins, presented a serious threat to public health as well as to animal economy. Large numbers of investigators and vast resources were drawn into investigations of the problem, and, as a result an enormous amount of literature in the field has accumulated during the past two decades. This large volume of literature is somewhat misleading by subtly suggesting a long-standing knowledge about mycotoxicoses. Actually, the field of mycotoxin research is barely out of its infancy and the public is becoming interested and involved in more of the ramifications of mycotoxicosis. Witness the public discussions of the "yellow rain" episodes in Southeast Asia and in Afghanistan (Marshall, 1982); irrespective of whether public pronouncements are based on established facts, such media presentations arouse scientific and general public interest and concern.

Surveys of food and feeds around the world have revealed that the problem of mycotoxicosis is not limited to any one geographic area but is a real or potential problem in all areas where molds grow (Shank, 1981). Virtually all staple food products consumed throughout the world are subject to contamination by mold toxins. Observations that some of the mycotoxins are carcinogenic in certain animal species and furthermore that they are associated with a high incidence of liver cancer in some human populations (UICC Technical Report No. 17) have added considerable impetus to research efforts. In addition, more recent data showing that environmental factors, including the diet and nutrients, can have significant effects on response to the toxins have served to increase further interest in the disease processes associated with mycotoxins. Means for preventing or alleviating such diseases are either at hand or under intensive investigation.

It is the purpose of this chapter to describe the influence of factors and conditions which modify the response of mammalian systems to mycotoxins with particular attention to effects of diet and nutrients. Only those mycotoxins for which there is evidence of human exposure and for defined adverse effects in man or in animals will be considered here. These will include the aflatoxins, where most information is available, the trichothecenes, and some data on ochratoxins and zearalenone.

3667

<u>Trichothecenes</u>

The trichothecenes (epoxytrichothecenes) are a group of fungal metabolites which possess antibiotic, antifungal, antiviral, phytotoxic, and/or cytotoxic properties (Ueno, 1977). The structures of representative members of this class are shown in Figure 1.

I = hydroxyl or acyl-substituted trichothecenes

II = 8-ketotrichothecenes

III = 7,8-epoxytrichothecenes

IV = verrucarins and roridins

V = 7,8-epoxyroridins

The acute toxicity of the trichothecenes are listed in table 1.

The fungi which produce these terpenoids (various Trichothecium, Cephalosporium, Myrothecium, and Fusarium species, among others) have been implicated in certain diseases of humans, animals, and plants (Ueno, 1977). Members of this group, most notably T-2 toxin, anguidine and nivalenol, have gained considerable visibility during the past two years as a consequence of allegations that human and animal populations are being exposed intentionally to fungal extracts containing these toxins in Southeast Asia (Marshall, 1982).

The 12,13-epoxytrichothecenes are powerful inhibitors of eucaryotic protein synthesis (Ueno and Fukushima, 1968; Shank, 1981; Bamberg and Strong, 1971). The trichothecenes inhibit DNA synthesis in a number of cell systems and in protozoa.

Trichothecenes have been subclassified according to the stage at which protein synthesis is interrupted (Bamberg, 1976; Liao et al, 1976). Specific inhibitors of the initiation, elongation, and termination stages have been identified. The initiation-type inhibitors (I) are generally more potent than the elongation (E) or termination (T) types (McLaughlin, et al 1977; Ueno, 1977). Cundliffe has suggested that a C.14 acyl substituent determines I vs. E or T activity (Cundliffe et al, 1974; 1977). Wei and McLaughlin (1974) however, have pointed out that C.15 hydroxylated derivatives can be effective initiation inhibitors if C.3 is substituted with an hydroxyl group.

Thus, T-2 toxin, nivalenol, and anguidine are type I inhibitors. Additional evidence points toward the eucaryotic ribosome, specifically the 60S subunit, as being the primary site of action of the trichothecenes. Although the trichothecenes possess antifungal properties, the fungi which produce these toxins are resistant to them. There are suggestions however that the resistance of Myrothecium verrucaria, a fungus which produces a range of trichothecene mycotoxins including T-2 toxin, may be due to the presence of ribosomes which are not subject to inhibition. In a more recent study Saccharomyces cerevisiae, a yeast normally sensitive to trichoderma, was transformed into a resistant strain by cloning with autonomously replicating plasmids carrying DNA fragments of the genome of a trichodermin-resistant yeast strain. It was concluded that trichodermin resistance in S. cereviriae is associated with ribosomal protein L3 (Busby and Wogan, 1981).

Information regarding the mechanism of action of the trichothecenes at the molecular level, however, is essentially non-existent. The minimum structural features required for biological activity appear to be the presence of the 12,13-epoxytrichothecene skeleton. Reduction of the epoxide leads to inactive derivatives while hydrogenation of the 9,10-double bonds leads to a substantial loss of activity. Rearrangement of the trichothecene skeleton to the apotrichothecene ring system or opening of ring C also leads to loss of activity. A C.4 hydroxyl or acyloxyl group is required for in vitro inhibition of peptidyl transferase (Doyle and Bradner, 1980). In the macrocyclic series of verrucarol derivatives, the macrocyclic ring must be intact for the roridins and verrucarins to have biological activity. Epoxidation of the 9,10-double bond or introduction of an allylic hydroxyl group at C.8 leads to an increase in potency.

It has been suggested that the mode of action of the trichothecenes involve nucleophilic additions to the epoxide group (Ueno, 1977). Trichothecenes appear to react with enzymes containing thiol groups at the active site (Ueno and Matsumoto, 1975). It is surprising, therefore, that T-2 toxin and 4-O-acetyl nivalenol (fusarenon-x) fail to react with glutathione and dithiothreitol in aqueous media. That these compounds are potent inhibitors of eucaryotic protein synthesis, however, does not necessarily mean that they react irreversibly with ribosomes.

Indeed, results presented by Liao and coworkers suggest that certain trichothecenes are reversible inhibitors (1976). These workers interrupted protein synthesis in Hela cells by treatment with five different trichothecenes. The cells were then washed with fresh serum, and were examined to determine whether protein synthesis resumed. Reversible inhibition of protein synthesis was observed with trichodermin and crotocin (elongation-termination inhibitors) but no reversal of the inhibition was observed by using T-2 toxin, anguidine and verrucarin A (initiation inhibitors). These data imply that the reversible inhibitors do not form covalent interactions with the ribosome.

The data for the "irreversible" inhibitors is equivocal, however. The data may imply that stable covalent interactions are established with the ribosome. On the other hand, the effect may simply be the consequence of very tight, non-covalent binding to the ribosome such that the trichothecenes are not easily washed off.

Relatively little work on the metabolism of the trichothecenes has been reported (Doyle and Bradner, 1980). T-2 toxin and acetoxynivalenol have been examined in a number of higher organisms including broiler chickens, mice, rats, among others (Ueno et al, 1971; Ueno, 1977). Anguidine went to Phase II clinical trials as an anticancer agent, but was subsequently abandoned; it has also been examined in microorganisms, and in dogs and monkeys (Anguidine, 1971), the latter only for toxic manifestations. The

only transformations documented thus far have been deacylation reactions of the trichothecene esters, which are for microsomal esterases. The relative potencies of partially acylated trichothecenes in the anguidine, nivalenol, and T-2 toxin families have been reported (Ohta et al, 1978; Claridge and Schmitz, 1978).

Mirocha and coworkers have reported detection of eleven metabolites of T-2 toxin in broiler chickens. Since there are at most seven compounds accessible from T-2 toxin by partial or complete deacylation reactions, at least four of these metabolites must be compounds which have undergone other chemical transformations in vivo. The structures of these compounds are not yet fully known, not is it known whether these metabolites are more potent or toxic than T2 toxin itself. Nonetheless, this evidence raises the question whether other trichothecenes including anguidine and nivalenol undergo chemical transformations in vivo (other than deacylation) to give biologically important metabolites.

Occurrence of Trichothecenes - Human and Animal Evidence for nutritional effects.

More than 40 trichothecenes, produced by various species of Fusarium, Trichoderma, Myrothecium, Trichothecium, Cephalosporium, Stachybotrys, Cylindrocarpon, and Verticimonosporium, have been studied extensively in association

with the toxicity of moldy cereal grains. Those currently known to exist fall into five structural categories as follows:

1. Hydroxyl- or acyl-substituted trichothecenes
2. 8-Keto trichothecenes
3. 7,8-Epoxytrichothecenes
4. Verrucarins and roridins
5. 7,8-Epoxyroridins

Structures and fungal sources of the naturally occurring trichothecenes are described by Busby and Wogan (1981). The fact that there are so many trichothecene mycotoxins is presumably because of the ability of trichothecene-producing Fusarium strains to acetylate or deacetylate exogenously supplied toxins, particularly deoxynivalenol and T-2 toxin and their derivatives.

Many of the naturally occurring trichothecenes, especially the verrucarins and roridins, are acutely potent toxins in mammals, with LD_{50} values in the 0.5 to 1.0 mg/kg range (Bamburg and Strong, 1971; Chi et al, 1977; Newberne and Rogers, 1981). Table 1 lists typical results of LD_{50} studies.

Vomiting (emesis) was often noted after dosing with the more toxic trichothecenes. Toxicity was primarily characterized by effects of the epithelial mucosa of the stomach and small intestine, resulting in mucosal erosion and severe hemorrhagic gastroenteritis. A radiomimetic component to trichothecene toxicity has also been observed in actively dividing tissues. This

3667

response is marked by necrosis and karyorrhexis of the thymus, testes, ovary, bone marrow, lymph nodes, spleen, and intestinal mucosa (Camargo et al, 1983). The trichothecenes are also potent skin necrotizing agents when applied topically.

There is very little information relative to the possible carcinogenicity of these important toxins. Rats ingesting a diet containing 10 ppm T-2 toxin over an 8-month period did not develop tumors; papillomas were not observed in mice after application of T-2 toxin and the cocarcinogen croton oil to the skin (Morases et al, 1969). Applying T-2 toxin to mouse skin with T-2 toxin or diacetoxyscirpenol over a 22-week period failed to produce lesions (Lindenfelser, 1974). On the other hand, however, acetoxyscirpendiol was eight times more effective than diacetoxyscirpenol in inhibiting the growth of transplantable mouse lymphatic leukemia (Claridge et al, 1978). As noted earlier, diacetoxyscirpenl was tested as an antitumor agent in clinical trials in the U.S. but results were disappointing and the trials were discontinued.

Although the disease associated with these toxins initially received many names, (septic angina, alimentary mycotoxicosis, alimentary hemorrhagic aleukia, aplastic anemia, hemorrhagic aleukia, and agranulocytosis), a committee of the Soviet Health Ministry concluded that the most appropriate term was Alimentary Toxic Aleukia (ATA).

At first the disease was considered to be infectious, but neither bacteriological studies nor epidemiologic investigations confirmed the hypothesis. Alternatively, the disease was considered a vitamin deficiency or a poisoning through bacterially contaminated food; neither of these hypotheses was substantiated. The various false concepts delayed recognition of the true nature of ATA. Eventually it was realized that the disease stemmed from ingestion of overwintered grains infested by toxic fungi. These grains formed the staple diet of the peasant population in agricultural areas of Russia.

ATA occurred with special severity during World War II, reaching a peak in 1944, when the population of the Orenburg district alone suffered an alarming number of casualties (Sarkisov, 1954). The morbidity among the population in this district exceeded 10% and a high mortality occurred in 9 of the 50 counties. Occurrence of the disease was related to the particular situation prevailing in some parts of the Soviet Union at that time; because of famine, the population was driven to collect grains that had been left in the field under the thick snow cover of winter. Under ordinary circumstances the wheat would have been gathered earlier but the shortage of manpower occasioned by the war prevented harvesting at the proper time.

The role of toxic fungi as an etiologic agent in ATA was examined extensively in the Institute of Epidemiology and Microbiology in Moscow. From more than 1000 samples of overwin-

3667

tered grains, the institute isolated over 3500 fungal cultures belonging to 42 genera and 200 species. Among the isolates, 61 were Fusarium poae and 57 Fusarium sporotrichioides, all of which were toxic to animals. Numerous investigations in different laboratories supported these results. Between 1943 and 1949, trial plot experiments determined the most favorable conditions for toxin production in overwintered wheat.

The extensive work conducted in the Soviet Union established that the disease was caused by toxic metabolites of species of Fusaria which grow on grains harvested after the snow has melted in the spring.

There appeared to be a nutritional component to the disease syndrome in humans. Populations receiving balanced diets were much less sensitive to the toxins than populations subsisting mainly on overwintered cereals (Newberne, 1974). Additional factors influencing the response to the toxins included the type of cereal ingested, the altitude, and the thorough washing of grains in boiling water before grinding. Prosomillet and wheat were the cereals most likely to be toxic. High altitude appeared to decrease the incidence of the disease in several population groups and the degree of toxicity was considerably diminished by washing the grains in boiling water which, as shown by later studies, removed some of the toxins.

In ATA, several investigators observed changes of the nervous system such as impaired reflexes, general hyperesthesia,

cerebral hemorrhages, encephalitis, and destructive lesions in the sympathetic ganglia.

The only prophylactic measure reported to date against ATA consists in the elimination of toxic grain from food.

Yellowed Rice Toxins

While there is no direct evidence to establish a relationship between the mycotoxins associated with the "yellowed rice syndrome," and nutritional status, there are suggestions that the intoxications in the 19th and early 20th centuries had a nutritional component particularly a deficiency of some of the vitamins. The "yellowed rice disease" described by the Japanese (Uraguchi, 1971) caused many human deaths. It was associated with the consumption of moldy rice imported from Southeast Asia. The Syndrome involved acute cardiac beriberi characterized by vomiting, ascending paralysis, convulsions and respiratory arrest. The initial diagnostic features in humans indicated that a thiamin (vitamin B_1) deficiency was likely acting in concert with the moldy rice toxins. However, the Japanese duplicated the disease in laboratory animals by dosing them with the toxin citreoviridin produced by Penicillium citreoviride, raising questions about real or potential nutrient involvement in the disease. Since the molds associated with the disease and the toxins they produce are widespread in the environment, a brief discussion is provided here.

It was shortly after World War II, when mold metabolites capable of inducing liver tumors in animals were found by the Japanese in domestic rice imported from Spain, Egypt, Thailand, Burma, Italy and the United States (Uraguchi, 1971). Several shipments were contaminated with a strain of Penicillium islandicum Sopp, the metabolites of which proved to be highly toxic;liver damage was the major manifestation. This recalled the episode among Japanese soldiers in World War II who developed edema of the legs in association with the consumption of rice contaminated with P. islandicum (Shank, 1976). This mold, capable of producing the nephrotoxic citrinin, was isolated from yellow rice imported from Thailand to Japan in 1951.

Although more than 15 kinds of fungi have been incriminated in moldy or yellowed rice, this review will cover only the most important or the best known types: P. islandicum Sopp, Penicillium citrinum Thom, and Penicillium citreoviride Biourge (Penicillium toxicarium Miyake).

The Japanese isolated P. islandicum Sopp in 1948 (Tsunoda, 1970). Tsunoda observed postnecrotic cirrhosis of the liver in rats fed for one month or less on rice contaminated with P. islandicum. The literature recounts the work that has since been done with P. islandicum by chemists, pathologists, clinicians, pharmacologists, mycologists, and others (Saito et al, 1971). Oral administration of a methanol extract taken from the fungus mat which had been cultured on Czapek solution for 14 days

induced severe liver damage in mice, mainly centrolobular necrosis and fatty degeneration. Further investigations revealed that the mold metabolites caused chronic liver damage, including cirrhosis and tumors, in rats as well as mice; the end result depended on the amount of moldly rice which had been consumed. According to severity of intoxication, the liver lesions were categorized as acute, subacute, and chronic (Miyake and Saito, 1965; Saito, 1970).

Acute intoxication, with atrophy of the liver, is caused by high levels of toxin given over a short period of time. Animals fed these high levels become inactive, progressively lose both muscular and cutaneous tone, and finally die after a prolonged comatose state similar to hepatic coma in man. Clinical pathology studies reveal several signs of liver damage. In human patients, histopathologic studies reveal mainly fatty degeneration and hemorrhagic centrilobular necrosis of the liver.

The subacute and subchronic intoxications, induced by lower concentrations of mold toxins over a longer period of time, cause moderate centrilobular necrosis with subsequent collapse of the stroma. These processes lead to fibrosis, liver atrophy, and bile duct proliferation. If the animal survives for a few weeks, the liver may show signs of regeneration.

Chronic intoxication develops in mice fed small to medium doses of moldly rice or mold metabolites. These mice survive the

early stage of intoxication without showing any signs or symptoms; they may survive for 6 months or more. Post-mortem examination reveals a wide range of liver damage from slight fibrosis and cell pleomorphism to cirrhosis and cancer. As with most other toxic conditions, a broad spectrum of clinical and histopathologic responses is observed.

The liver lesions, including tumors have been described by Saito et al (1971) and by Enomoto (1959). Histologically, the changes range from mild parenchymal cell hyperplasia to differentiated and undifferentiated liver cell carcinoma. Although there is a high incidence of liver injury, the incidence of malignant parenchymal tumors is relatively low, indicating a low carcinogenic potential for metabolites of P. islandicum Sopp.

Among laboratory animals, rabbits are the most susceptible species in regard to metabolites of P. islandicum Sopp (Saito et al, 1971); when fed 1-5% moldy rice, most died within a few days. Those that survived for longer periods developed postnecrotic cirrhosis as soon as 90 days after the initiation of feeding studies. Although rhesus monkeys also showed acute toxic damage of the liver, they developed neither cirrhosis nor tumors as end results of intoxication with metabolites of P. islandicum.

Diet profoundly affects the responses of animals to toxins of P. islandicum (Saito et al, 1971). Both male and female mice develop acute toxic effects in a short period of time when fed

rice infected with P. islandicum, but the response is greatly enhanced by low protein intake, as illustrated by the following example. The death rate of mice during three weeks of feeding with an 11% protein diet containing 3% moldly rice was about 44% in males and about 25% in females. The death rate of mice fed the same percentage of moldly material with a 34% protein diet was 28% in males and 5% in females. As is the case with many other liver toxins and carcinogens, the male was considerably more sensitive.

Organs other than the liver are also injured by exposure to toxic metabolites of P. islandicum (Ishiko, 1957; Miyake, 1960). Lesions include atrophy of the thymus, spleen, and fat tissues, fatty degeneration of renal epithelium, and pancreatic fibrosis occasionally develops in mice and rats. In addition, tumors originate in tissues other than the liver.

One of the toxic agents isolated from the media in which P. islandicum Sopp was grown received the name luteoskyrin (Kobayashi, 1958). The material was obtained from the media as one of seven pigments, including those later named rugulosin and cyclochlorotine; the latter has also been referred to as islanditoxin (Saito et al, 1971).

Toxins associated with "yellowed rice," in addition to luteoskyrin, include cyclochlorotine, produced by P. islandicum, citreoviridin, produced by P. citroviride, and Rugulosin, produced by P. rugulosum.

3667

Toxicity of luteoskyrin varies with the route of administration (Saito et al, 1971). In mice, the LD_{50} (mg/kg body weight) is as follows: intravenous, 6.65; intraperitoneal, 40.8; subcutaneous, 147; and oral, 221. Repeated subcutaneous injection of less than 1/10 of the LD_{50} over several days produces the same lethal effect as a single subcutaneous LD_{50} but requires a longer time. Very young mice are more sensitive than older ones, and males are more sensitive than females.

The pathologic effect of luteoskyrin resides primarily in the liver, being similar in rats, mice, rabbits, and monkeys. The macroscopic changes of yellow discolored liver can be seen within 24 hr after exposure. Marked centrilobular necrosis and fatty degeneration occur, with some nuclear pleomorphism and hyperchromatosis. Following prolonged exposure, mice develop liver tumors but cirrosis has not been reported.

The pathologic response to rugulosin reportedly is almost identical with that to luteoskyrin.

Although the toxins luteoskyrin, islandotoxin, and cyclochlorotine share many pathological effects, their biological effects are quite different. Luteoskyrin and islandotoxin cause liver damage characterized by centrolobular necrosis. Cyclochlorotine, on the other hand, causes damage in the peripheral zone, characterized by vacuolation of liver and endothelial cells and the appearance of hyaline droplets in the

cytoplasm. A similar cytotoxic effect results from cysteine deficiency or from allyl formate administration.

Long-term feeding studies with cyclochlorotine and luteoskyrin in mice have shown that luteoskyrin is a cirrhogenic agent and may be carcinogenic, although this aspect requires further study; in limited experiments, a few mice have developed liver tumors.

Citrinin is the major yellowed-rice toxin produced by P. citrinum; its chemical structure has been established (Mathieson and Whalley, 1964) and its biosynthesis accomplished.

The LD_{50} of citrinin has been reported for mice, rats, rabbits, and guinea pigs; the subcutaneous LD_{50} varies among these species from 35 to 67 mg/kg body weight. Intraperitoneally, the LD_{50} is 30 mg/kg in mice and about 50 mg/kg in rabbits.

Besides renal damage, citrinin causes acetylcholine or pilocarpine-like responses, including vasodilation, constriction of the bronchi, and increased muscular tone.

An additional mold associated with yellowed rice was isolated several years ago from rice collected in Taiwan and Japan. Designated as Penicillium toxicarium Miyake, it was later shown to be identical with Penicillium citreoviride Biourge, which had been described earlier by two laboratories (Saito et al, 1971).

3667

Feeding studies proved the contaminated rice to be toxic to rats. Rice infested with P. citreoviride was studied for its toxicologic effects; more recently, an active fraction has been isolated, chemically identified (Saito et al, 1971) and designated as citreoviridin.

The toxicology of citreoviridin is known for several mammalian species. It causes an acute poisoning characterized by an early onset of progressive paralysis in the hindlegs, vomiting, convulsions, and a respiratory disorder. At an advanced stage, cardiovascular disturbances, flaccid paralysis, and hypothermia occur along with dyspnea, gasping, and coma; respiratory arrest and death follow. The symptoms in animals resemble closely those in human patients suffering from acute cardiac beriberi - also called shoshinkakke- a common disease throughout Asian countries during the recent past. The cause of this type of beriberi remains unknown; there appears to be ample thiamine in the tissues of patients. In both man and animals, the disease is characterized primarily by ascending progressive paralysis. A nutritional effect is apparent however which is likely a result of inhibition of utilization of thiamine.

Despite the very severe clinical symptoms attributable to citreoviridin, histopathological changes are minimal or not detectable at all.

Zearalenone

Zearalenone is a phenolic resorcyclic acid lactone (fig. 2). It is classified according to biosynthetic origin as a nonaketide within the group polyketides. It possesses potent estrogenic properties. The compound was recovered originally from cultures of Gibberella zeae, the sexual stage of Fusarium roseum. These cultures had been isolated earlier from moldy corn associated with a hyperestrogenic syndrome in pigs (Stob et al, 1962; Pathre and Mirocha, 1976; Christensen et al, 1965). Feed contaminated with zearalenone caused uterine enlargement and swelling of the vulva, mammae and nipples in prepubertal female swine. Swollen mammae and testicular atrophy occurred in young male swine (Mirocha and Christensen, 1974; Mirocha et al, 1977). Zearalenone is synthesized by strains of Fusaria including F. graminerum, F. tricinctum, F. oxysporum, F. sporotrichiodes, and F. moniliforme. A period of low temperature (12°-14°C) is required for significant toxin formation.

Zearalenone occurs as a natural contaminant in corn, wheat, barley, oats and sorghum (Mirocha et al, 1977; Ueno, 1973). In a (1972) Food and Drug Administration survey for zearalenone contamination about 17% of 223 corn samples contained levels ranging from 0.1-5.0 ppm (Eppley et al, 1974). It has been found as a contaminant in corn in England, Finland and Yugoslavia and in beers from Zambia (Lovelace and Nyathi, 1977) and Swaziland (Martin and Keen, 1978).

The acute toxic effects of zearalenone and its active derivatives, if indeed these can be considered "toxic," appear to be

limited to hyperestrogenism and its sequelae (Mirocha et al, 1977). Table 2 lists levels of contaminants occurring naturally which have been associated with hyperestrogenism in swine. In field cases, zearalenone contaminated feed is associated with stillbirths and splayed legs in swine (Miller et al, 1973) but controlled experiments (Patterson et al, 1977) failed to confirm the earlier observations.

Studies in rats however, confirmed an association of zearalenone with decreased fertility, resorptions and stillbirths in animals given 10 mg/kg body weight daily (Bailey et al, 1976). Zearalenone has produced a dose dependent increase in the incidence of skeletal anomalies in rat embryos (Ruddick et al, 1976). The few reported studies relative to mutagenicity have been negative or, if positive the data have been unconvincing (Wehner et al, 1978; Kuczuk et al, 1978; Ueno, 1977).

There are no reports in the literature relative to the effects of nutrients or nutritional status on the response of animals to zearalenone or its active derivatives. The only accessible data are from some preliminary studies (unpublished) conducted in our own laboratory. Mice of the CD-1 strain were fed either a diet deficient in vitamin B_6 or a diet supplemented with adequate vitamin B_6. Half of each group were given 100 micrograms orally of zearalenone three weeks after the diet was initiated and uterine weights recorded two weeks later. The deficiency prevented uterine enlargement, compared to the control group treated with zearalenone only.

Zearalenone and its estrogenic congeners are true estrogens and their physiological effects appear to be limited to such effects.

The Ochratoxins

The ochratoxins are a group of seven compounds, structurally related as shown in fig. 3. They are isocoumarin derivatives linked with an amide bond to the amino group of L-B- phenylalanine. Ochratoxin A is the most toxic of the group and, most of the literature deals with this member of the group (Harwig, 1974; Steyn, 1977).

Ochratoxins are produced by at least seven species of Penicillia and six species of Aspergillus. The major producers of the ochratoxins are P. cyclopium, P. viridicatum and P. palitans (Krogh, 1977).

The ochratoxins have been identified in peanuts, corn, wheat, oats, barley and rye. They cause a nephropathy in pigs which is endemic in Denmark (Krogh, 1977). The disease occurs in swine in Europe and North America. Poultry also are affected with ochratoxin-induced nephropathy which results in a relatively high rate of condemnation by meat inspectors because of the renal lesions (Elling et al, 1975). Ochratoxin residues up to about 70 ug/kg have been detected in kidney, muscle and fat of pigs and in muscle meat of poultry.

The acute toxicity of ochratoxin is listed in table 3. Experimental infection with ochratoxins affect primarily the kid-

ney and to a lesser extent, the liver. A dietary level of 200 ppb over a 3 to 4 month period produced typical nephropathy.

Ochratoxin A is acutely toxic to mammals and birds, as noted in table 3, with synergism between ochratoxin A and penicillic acid or citrinin (Harwig, 1974).

Ochratoxin A is teratogenic to mice, rats and hamsters producing both skeletal and soft tissue defects. There is no convincing evidence that ochratoxins are carcinogenic (IARC, 1976) but one report from Japan (Kanisawa and Suzuki, 1978) suggests that 40 ppm of ochratoxin A in the diet may induce hepatic and renal adenomas.

There are no published reports for mutagenic activity of any of the ochratoxins.

There is a striking similarity in the nephropathy produced in animals by ochratoxins and the endemic nephropathy (Balkan nephropathy) found in rural populations in Bulgaria , Romania and Yugoslavia (Puchlev, 1974). This disease results in remarkably reduced size and function of kidneys; it is most common in women, and is slowly progressive to death. Epidemiological evidence of geographical distribution of the disease and from a survey of foodstuffs suggests that ochratoxin A and the prevalence of Balkan nephropathy are correlated in one area of Yugoslavia (Krogh _et al_, 1977).

There are no reports in the accessible literature for a nutritional effect on response of animals to ochratoxins.

Aflatoxins

Although Aspergillus flavus is associated with most food and feed contaminations with the aflatoxins only a few strains of A. flavus actually produce the seconday metabolites, classified under the generic term aflatoxins. These few strains are the most important producers of aflatoxin but other Aspergilli also produce aflatoxins but in much smaller amounts (Sargeant et al, 1961; 1963).

The majority of the more than 2000 publications about aflatoxins which have appeared during the last 20 years, reflect an intense interest in mycotoxins, and more specifically, in aflatoxins. This interest was initially aroused by the outbreak of turkey X disease throughout England in 1960 (Sargeant et al, 1961).

During the early development of antibiotics, a fundamental discovery was made that some materials from antibiotic-producing molds were toxic in animal trials. These molds were usually discarded because they were poor producers of antibiotics and little attention was paid to their potential for producing toxins. Thus, the recognition of mycotoxins as a threat to public health was delayed for two or three decades.

3667

It has long been known that fungi growing on foods and feeds are ubiquitous, and that they may contaminate virtually every

staple foodstuff of either plant or animal origin. The majority of them grow well within extremely wide ranges of pH and temperature, making moisture conditions the primary environmental restriction. On cereal grains and oil seeds, molds grow at moisture levels which are commonly encountered under storage conditions. These storage molds are mainly species of Aspergilli and Penicillia. As only one example, moldy corn toxicosis in swine, occurs with considerable frequency in the humid southeastern parts of the United States, and is associated with both Aspergillus and Penicllium molds (Burnside, 1957).

Aspergillus molds were incriminated in the epizootics of bovine hyperkeratosis, although later studies indicated that the disease was more complex; it appeared to result from an interaction of chlorinated cyclic hydrocarbons and nutritional factors, particularly vitamin A. (Hansel et al, 1951; Sikes and Bridges, 1952; Hoekstra et al, 1954). Forgacs and Carll (1955) isolated several molds, mostly species of Aspergilli and Penicillia, from feeds which produced a hemorrhagic disease in poultry. Some of these were capable of reproducing the hemorrhagic syndrome in birds. These diseases were then designated as moldy feed toxicoses.

The English episode in turkeys brought together microbiologists and veterinarians who quickly recognized that the toxic manifestations were caused by the ingestion of certain mold-contaminated feeds.

During the period from 1960 to 1962, several reports appeared (Sargeant et al, 1961; Wolf and Jackson, 1963) about outbreaks of disease in poultry and fish at diverse geographical locations. Fungi in feeds were suggested to be the likely etiologic agents. In avian species, the acute disease resulted in loss of appetite, weakness of wings, and lethargy. Histologic examination of tissues revealed an acute hepatic necrosis and a marked bile duct proliferation. Ultimately, the problem was traced to imported Brazilian peanut meal in the birds' rations, referred to as Rosetti meal because the name of the transport vessel was Rosetti. It was then discovered that ducklings, swine, and cattle had also been poisoned by similarly contaminated lots of peanut meal. The Brazilian peanut meal, however, was not alone in causing toxicity. At about the time that the turkey X disease broke out, a similar disease associated with peanuts processed in East Africa, affected ducklings in Kenya (Asplin and Carnaghan, 1961). The early discovery that ducklings were particularly sensitive to aflatoxin, as evidenced by a rapid and extensive bile duct proliferation, was fortuitous; this species became the major tool for biologic assay. Following the initial studies of peanut meal poisoning in turkeys and ducklings, Lancaster et al (1961) reported that rats developed liver cell carcinomas when toxic peanut meal was included in their diet for 30 weeks or longer. Sargeant et al (1961) and Nesbitt et al (1962) identified the toxin-producing organism in the peanut meal as the saprophytic mold Aspergillus flavus Link

3667

ex Fries, a widely distributed mold that has been isolated from virtually every staple food product in the tropical and semitropical areas of the world (Wogan, 1973).

While the turkey X disease was at its height, an epizootic of liver cancer afflicted hatchery-reared rainbow trout in Washington and Oregon. Investigations then revealed that liver carcinoma occurred in fish at many different locations. Trout raised in hatcheries had for many years been fed a diet including vegetable sources of protein with cottonseed meal as the major source (Sinnhuber, 1968). As a result of many investigations, the etiology of trout hepatomas was finally traced to contaminants in the cottonseed meal. Furthermore, the investigators discovered the influence of nutritional factors on both the neoplastic processes (Sinnhuber, 1968) and the resistance to carcinogenic toxins.

In addition to toxicoses in various domestic animals and liver carcinomas in trout, an observation in laboratory rats further heightened the interest in contaminated feed. During studies comparing the effects of choline-deficient and adequate diets, Salmon and Newberne (1963) observed liver carcinoma in rats fed diets containing peanut meal and adequate levels of choline. Within a relatively short time, several reports (Newberne et al, 1964; Newberne, 1965) showed that the contaminant in peanut meal was the same as that produced by a strain of Aspergillus flavus, isolated from toxic meal in England. The

contaminants were identified as a complex of metabolites produced by Aspergillus (Asplin and Carnaghan, 1961; Loosmore and Markson, 1961; Allcroft and Carnaghan, 1963).

Identification and Characterization of Aflatoxins

The discovery that the toxins had a characteristic fluorescence pattern on thin-layer chromatograms greatly facilitated their isolation and characterization, culminating in the determination of the molecular formulas of four components designated as aflatoxins B_1, B_2, G_1, and G_2; the four components were distinguished by their blue or green fluorescence and by their R_f values on thin-layer chromatograms (Asao et al, 1965). More recently, related substances have been isolated and chemically characterized, the most important ones being M_1, M_2, B_2, and G_{2a} (Wogan, 1973). The M_1 and M_2 fractions, first isolated from the milk of cows feeding on aflatoxin-contaminated fodder (Allcroft and Carnaghan, 1963) were later found in the milk of laboratory rats. M_1 and M_2 possessed the same toxicity as the aflatoxins from which they were derived (Purchase, 1967). On the other hand, the hydroxylated B_{2a} and G_{2a} derivatives of B_2 and G_2 described by Dutton and Heathcote (1968) were virtually non-toxic, suggesting that contaminated foods may be detoxified by acid treatment.

The isolation, purification, and identification of the various fractions of aflatoxins have been described in detail (Detroy et al, 1971). The structures are shown in Figure 4 and metabolic pathways described (Busby and Wogan, 1984).

The biosynthesis of aflatoxins has been studied extensively. In the earlier work (Adye and Mateles, 1964) various precursors were labeled and tested for incorporation into the synthetic pattern of aflatoxin B_1. Phenylalanine and shikimic acid appeared implicated as precursors (Donkersloot et al, 1968). Although several hypothetical schemes were devised, the precise nature of the biosynthesis remains to be established. On the other hand, procedures for laboratory synthesis of aflatoxins B_1, G_1, and M_1 have been developed (Buchi and Weinreb, 1969). The syntheses were designed primarily for structural verification. The procedures are too complex for routine production of material for experimental use.

A total of 12 structurally related compounds with similar configurations have been identified; Buchi and Rae (1969) described in detail both the structure and chemistry of many of these compounds. Products of metabolism in the biological systems also were investigated. These superb chemists continue to elucidate structures, the activity of which provide challenges for toxicologists. Following isolation, purification and availability of pure material, many studies have demonstrated the dose response characteristic of AFB_1 illustrated in table 4.

Numerous reports have dealt with factors of various types that modify the carcinogenic and other toxic effects of mycotoxins in experimental animals. These include sex-linked and endocrine characteristics, and interactions with other environ-

mental factors. The effects of nutrients deserve particular attention in view of nutritional deficiencies occurring in those parts of the world, where aflatoxin exposure is most prevalent. The more recently discovered effect of exposure to (artificial) sunlight is also of interest in this respect since the metabolism of these compounds may be affected by percutaneous exposure to UV irradiation (Newberne _et al_, 1974; Joseph-Bravo _et al_, 1976).

The mechanisms by which hormones, nutrition, and other factors influence aflatoxin carcinogenesis are not known. They appear to include effects on DNA synthesis, cell division and differentiation, and/or effects on aflatoxin metabolism, and excretion. Animals with a severely restricted food energy intake do not grow and are less susceptible to the action of many carcinogens, compared to normal animals. The retardation of growth induced by severe protein deficiency, by hypophysectomy or otherwise may explain reduced tumour incidence under these experimental conditions. Conversion of aflatoxin B_1 to a bacterial mutagen is different in microsomal liver preparations from rats fed a marginal lipotrope diet compared with those from normal rats. Excretion of mutagens in the urine is also different in lipotrope-deficient rats (Suit _et al_, 1977).

Changes in aflatoxin B_1 metabolism and reduced levels of hepatic macro-molecule-bound aflatoxin B_1 adducts were reported in rats pretreated with phenobarbital (Garner, 1975; Swenson _et al_, 1977), and in hypophysectomized animals (Swenson _et al_,

1977). Dietary factors (vitamin A, protein, lipotropes) referred to later, also modulate AFB_1 metabolism, in part through effects on enzyme systems.

Sex-linked differences and endocrine status. Results of a number of studies indicate that in comparison with males, female rats are more resistant to both acute toxic and carcinogenic effects of aflatoxins. A single administration of aflatoxin B_1 by gavage produced a calculated LD_{50} of 7.2 mg/kg body weight (fiducial limits 5.36-8.23) in male rats and 17.9 mg/kg body weight (fiducial limits 14.4-22.5) in female rats (Butler, 1964). The sex-dependent influence of vitamin A deficiency on the acute toxicity of aflatoxins is discussed later in this section.

In another study (Newberne and Wogan, 1968a), Fischer rats of both sexes were kept on diets containing aflatoxin B_1 levels of 0.015, 0.3, or 1.0 mg/kg and killed for histological examination at sequential periods over a lifetime. The early hepatic lesions, considered by some to be precancerous, appeared with almost the same rate of incidence and at approximately the same time in both sexes; however, there was a considerably longer period between the appearance of the precancerous lesions and progression to liver carcinomas in females compared to males. At a dietary level of aflatoxin B_1 of 1 mg/kg of diet, males developed carcinomas after 35 weeks of exposure while tumors in females were observed only after 64 weeks. A similar, but much less pronounced, sex-linked difference was observed at the 2 lower

aflatoxin levels. Calculations of the approximate total intake of aflatoxin B_1 before the appearance of tumours (based on average intake of food containing a known quantity of aflatoxin) and the time over which these total amounts were consumed are shown in Table 4. In a study by Ward et al (1975), male rats (F344, NIH) kept on a diet containing aflatoxin B_1 at 2 mg/kg, died with malignant haemorrhagic liver tumours significantly earlier than females. Kidney tumours were observed in male but not female rats exposed to aflatoxins (Butler et al, 1969).

Newberne and Williams (1969) reported that fewer male rats (Charles River CD) fed for more than a year on a diet containing aflatoxin B_1 at 0.2 mg/kg and diethylstilbestrol at 4 mg/kg developed liver tumours (8/40) than those fed the same aflatoxin diet without the estrogen (25/35), indicating an important sex hormone effect.

In a study on the influence of hypophysectomy on aflatoxin carcinogenesis, male albino (MRC) rats were fed a diet containing an aflatoxin B_1 level of 4 mg/kg. Eleven of 14 control rats developed liver tumours in 49 weeks whereas none of 14 hypophysectomized rats developed liver tumors in the same period. However, tumors of extrahepatic tissues (4/14 carcinomas of retro-orbital lacrimal glands were observed in the hypophysectomized aflatoxin-treated rats (Goodall & Butler, 1969).

3667

NUTRITIONAL FACTORS (FOOD COMPONENTS). The influence of

nutritional factors on the effects and dose-response relationships of aflatoxins has been reviewed by Newberne (1974, 1976), Newberne and Rogers (1976), Newberne & Gross (1977); and Newberne, (1984). The more important nutrient effects will be discussed in the following paragraphs.

(a) Dietary protein and lipotropic agents. Some interesting data have come from Rhesus monkeys which were given daily doses of 100 ug aflatoxin per animal by stomach tube. Two animals, fed a severely protein-deficient ration (1% casein) for 8 weeks before and during aflatoxin administration, developed fatty liver, bile duct proliferation and fibrosis, and died with gastrointestinal haemorrhage within 30 days of aflatoxin treatment. Two animals fed a control ration (16% casein) survived in apparent good health up to the termination of the experiment (35 days of aflatoxin treatment) (Madhavan et al., 1965a).

In a study on weanling male rats given 50 ug aflatoxin per animal per day for 20 days, 2/6 animals, fed a diet containing 4% casein, died on days 18 and 19 of the experiment. Extensive liver damage was found in these rats and in others in the 4% casein group within 20 days of aflatoxin treatment. All 12 rats fed a diet containing 20% casein survived similar aflatoxin dosing with only mild changes in the liver. In another study by Madhavan & Gopalan (1968) 2 groups of 12 male rats were fed the 2 diets (5% or 20% casein) for 2 years from weaning and were given, from the beginning of the experiment, 232 daily doses of 5 ug

aflatoxin per animal or 225 daily doses of 10 ug aflatoxin per animal. All 12 animals fed the 20% casein diet survived the period of aflatoxin dosing and 50% developed hepatomas; lung metastases were observed in 2 of these rats. Five of the 12 animals fed on the 5% casein diet died during the period of aflatoxin dosing. No hepatomas but one renal cell carcinoma were found in the remaining 7 rats. Both diets used in these experiments were supplemented only with 0.01% choline (Madhavan and Gopalan, 1975).

In experiments by Newberne & Wogan (1968b), rats fed a diet containing 9% protein developed a higher incidence of liver tumours (11/15) in a shorter period of time (8 months) than rats fed a diet containing 22% protein (incidence 7/14 after 10 months). Both groups of rats were given a total dose of 375 ug of aflatoxin B_1 per animal by gastric intubation over 3 weeks, at the beginning of the experiment.

In studies examining lipotropic effects (methionine, choline, folate and vitamn B_{12}) on aflatoxin activity, a diet marginal in methionine and choline, deficient in folate, and high in fat protected male rats against the acute toxicity of a single dose of aflatoxin B_1; however, susceptibility to the toxic effects of repeated doses of aflatoxin B_1 increased, and the carcinogenicity of aflatoxin B_1 was enhanced. The experimental diet (Rogers & Newberne, 1971) contained peanut meal (12%, alcohol extracted), gelatine (6%), casein (3% vitamin free) and fibrin

3667

(1%) as sources of protein, with a supplement of L-cystine (0.5%). This diet is marginally but not severely deficient in threonine, tryptophan, and arginine as well as in methionine. The diet contained choline chloride (0.2%), and was high in fat (beef fat 30%; corn oil 2%). The control diet contained casein (22%, vitamin free) as the protein source, 15% or 16% oil (corn or mixed vegetable oil) and 0.3% choline chloride. Both diets were adequate in other essential nutrients.

The marginal lipotrope diet fed for 2 weeks protected rats against acute aflatoxin B_1 toxicity; those that died had hemorrhagic necrosis of the liver with varying degrees of bile duct proliferation and, some had hemorrhagic necrosis in the adrenals and kidneys. The surviving rats (killed 2 weeks after aflatoxin administration) had focal necrosis of liver parenchymal cells. Approximately 20% of the rats fed the marginal lipotrope diet and given a single dose of aflatoxin B_1 had focal areas of abnormal hepatocytes which had increased uptake of ^3H-thymidine (Rogers & Newberne, 1971).

Lipotrope deficient rats are resistant to the toxicity of a single dose of aflatoxin B_1, but they are sensitive to repeated aflatoxin exposure. One half of 136 male rats died, most of them after receiving 8 or 9 doses of aflatoxin B_1 (200 or 225 ug total). Various degrees of necrosis were observed in the livers with extensive proliferation of bile duct cells. The mortality in rats fed the control diet (66 males) was only 4% during the

administration of the total dose of 350 ug of aflatoxin B_1 (Rogers and Newberne, 1971).

The enhanced aflatoxin B_1 carcinogenicity in rats fed marginal lipotrope diets, have been reviewed by Newberne & Gross (1977) and more recently by Rogers, 1975; Newberne et al 1982; Rogers and Newberne, 1980. Figure 5 illustrates the cumulative probability of death from a tumor, calculated here by the method described by Saffiotti et al. (1972) i.e., from the number of animals at risk and the number of deaths from tumours each week. The rats were fed either a complete diet (diet 1) or a diet low in lipotropes (diet 2) and given a total dose of aflatoxin B_1 of 375 ug, over 3 weeks. Hepatocarcinomas developed in 87% of 52 animals fed the marginal lipotrope diet and in only 11% of 27 rats fed the nutritionally complete diet ($P<0.001$). Twenty-seven percent of tumours in rats fed the marginal diet metastasized to other abdominal organs or the lung; no metastases were detected in rats fed the control diet (Rogers, 1975). The peanut meal fed in these experiments was assayed and did not contain detectable aflatoxins. Similar effects have been observed with other carcinogens (AAF and DEN).

Further studies were carried out to determine how far the high fat content of the marginal lipotrope diet contributed to the enhancement of carcinogenesis. Male Fischer rats were fed the marginal lipotrope diet, the nutritionally complete contiol diet, or the control diet with substitution of the fat from the

marginal lipotrope diet (30% beef fat, 2% corn oil) for the fat in the control diet (15% mixed vegetable oils). Each rat was given a total of 375 ug of aflatoxin B_1 intragastrically over 3 weeks and allowed to live until moribund or dead, or until 90 weeks after treatment, and then necropsied. Hepatocarcinoma incidence was based on the number of rats that survived until the first death with hepatocarcinoma, i.e., 27-34 rats per group. Tumors were found in 39% in rats fed the marginal lipotrope diet, 15% in control rats, and zero in rats fed the control diet with beef fat (30%) and corn oil (2%) substituted for vegetable oil (15%). Thus under these conditions the high fat content of the deficient diet inhibited, rather than contributed to the enhancement of aflatoxin carcinogenesis. It appears that the fat is of less consequence than the lipotropes.

An enhancing effect on aflatoxin carcinogenicity was observed in experiments in which male rats (Charles River CD Sprague-Dawley) were fed a low lipotrope diet containing 20% isolated soybean protein and supplemented with 0.1% DL-methionine and 0.1% choline chloride. Aflatoxin B_1 was given intragastrically during the early weeks of the experiment, a total dose of 240 ug/animal (divided in 24 daily doses of 10 ug, 5 days a week). Liver cell carcinomas were observed in 5/17 animals fed this diet. No tumours were found in rats given the same dose of aflatoxin B_1 and fed the same basal diet supplemented with 0.6% DL-methionine, 0.6% choline chloride, and vitamin B_{12} supplement (Newberne et al., 1968c).

A severe, rather than a marginal lipotrope deficiency may decrease rather than increase the incidence of liver carcinoma in aflatoxin-treated rats. A decrease in liver carcinoma was observed in aflatoxin-treated, male, Sprague-Dawley rats with severe lipotrope deficiency particularly if penicillin, at a level of 0.1% were added to the diet. The expected penicillin-induced inhibition of cirrhosis was not observed and the interactions between penicillin and aflatoxins have not been elucidated. The diets used in these experiments, (the control, adequate, and the severe lipotrope-deficient) contained alcohol-extracted groundnut meal (25%) and casein (6%) as the protein source. No cystine or methionine was added; choline and vitamin B_{12} were added to the control diet at levels of 0.3% and 50 ug/kg respectively. When rats were killed 12 months after receiving a total dose of 375 ug aflatoxin B_1 per animal (divided into 15 daily intragastric doses of 25 ug each per animal), hepatomas were found in 64% (9/14) control animals and in 21% (7/17) lipotrope-deficient animals. In rats fed diets containing penicillin, hepatomas were found in 70% (14/20) of the controls and in only 17% (3/18) of the lipotrope-deficient animals (Newberne & Rogers, 1971).

Vitamin B_{12} has minimal lipotropic characteristics. It has recently been found in concert with protein to increase tumor incidence in aflatoxin-treated rats. In a study by Temcharoen et al. (1978) repeated later, (Temcharoen, 1983) male Fischer rats

were fed diets containing 20% or 5% casein with or without vitamin B_{12} (50 ug/kg diet) for 33 weeks. Table 5 illustrates such effects. The diets were similar to those used in previous studies by Wogan & Newberne (1967) and Newberne and Wogan (1968a) which contained 0.036% choline chloride. The casein content was similar to that in diets used in the study by Madhavan & Gopalan (1968) mentioned earlier. A mixture of crystalline aflatoxins (containing aflatoxins B_1 and G_1 approximately in the proportion of 1:1 and about 5% of aflatoxins B_2 and G_1) was added to the diets at the level of 1 mg/kg. As shown in Table 5, vitamin B_{12} supplementation increased liver tumor incidence in rats fed a diet containing aflatoxin and 20% casein. Severe protein deficiency affected the growth of the animals; the body weight of rats fed 5% casein was reduced to about one third of the controls at the termination of the experiment. Liver cirrhosis was observed only in the aflatoxin-treated, protein-deficient rats. As suggested by Temcharoen et al. (1978), a high incidence of hyperplastic nodules and cholangiofibrosis in the protein-depleted, aflatoxin-treated animals may indicate that the carcinogenic process was retarded but not eliminated.

(b) Vitamin A. In a study by Reddy et al. (1973), male and female albino rats fed a vitamin A-deficient diet for 9 weeks after weaning or fed the same diet with a daily oral supplement of 30 ug (100 IU) of vitamin A/rat were given a preparation of crystalline aflatoxins containing aflatoxins B_1 (44%), G_1 (44%),

and B_2 and G_2 (2%) in a single-intraperitoneal dose of 3.5 mg/kg body weight. High mortality was observed in vitamin A-deficient males (Table 6). The vitamin A-deficient females and all the vitamin A-supplemented animals had no adverse reactions 40 h after aflatoxin injection. Histologically, severe liver damage was observed in vitamin A-deficient, aflatoxin-treated male rats, in contrast to minimal liver damage in female rats and male rats given vitamin A.

In a study conducted in our laboratories on rats fed diets containing aflatoxin B_1 in the range of 15-100 ug/kg and deficient, adequate or excessive in vitamin A over a 2-year period, the vitamin A-deficient animals developed a similar incidence of liver tumours to the other 2 groups but had an increased incidence of colon carcinomas (Newberne & Rogers, 1973). Thus, in a group of 50 male rats (Charles River CD Sprague-Dawley) exposed to a dietary level of aflatoxin B_1 of 100 ug/kg and 5 ug vitamin A (retinyl palmitate) per animal per day, colon tumours and liver tumours were observed in 6 and 11 rats, respectively, whereas with daily intakes of 50 or 500 ug retinyl palmitate per rat, no colon cancers were observed and the incidence of liver tumours was 24/50 or 19/50, respectively. The results of a further study (Newberne & Suphakarn, 1977) are shown in Table 7. Charles River CD Sprague-Dawley rats were fed a diet containing aflatoxin B_1 at a level of 1 mg/kg (AFB_1) and 3 different dietary levels of vitamin A (retinyl acetate). Again, there was an increased incidence

3667

of colon carcinomas in vitamin A-deficient rats. Excessive vitamin A did not protect against aflatoxin carcinogenesis in either the liver or the colon.

Certain mechanistic aspects of aflatoxin-induced colon cancer in rats have been elucidated. We have continued studies relative to possible mechanisms with special emphasis on glutathione (GSH) conjugation, enzyme activities in liver, intestinal and colon mucosa; and binding of AFB_1 to colon epithelial DNA. The following highlights of the results, in print elsewhere (Suphakarn et al, 1983) are presented here in the context of nutritional influences on aflatoxin toxicity and carcinogenicity.

Male weanling (21-day-old) Sprague-Dawley rats, 45 $\pm$ 5 g body weight were pair-fed a semisynthetic diet containing either 2.0 ug retinyl acetate per g diet (adequate vitamin A) or containing no added vitamin A. After 7-8 weeks on the diets, the vitamin A-deprived rats exhibited deficiency symptoms including plateau of weight gain, rough hair coat, and lowered serum vitamin A content. At this point the deficiency was marginal and the vitamin-A-deprived rats were given retinyl acetate (3 ug/rat) in cottonseed oil, by gavage, once weekly for the remainder of the 9 weeks of the experiment, while the various assays and analyses were completed.

To test the effect of marginal vitamin A (3 ug vitamin A/wk) on enzymes and other parameters after exposure to AFB_1, the car-

cinogen, was administered to both experimental and control rats for 5 successive days (25 ug/day; total of 125 ug/rat). On completion of the dosing schedule, the following determinations were made: microsomal protein; the microsomal enzymes benzo(a)pyrene hydroxylase (BPOH), p-nitroanisole-o-demethylase (PNA), and B-glucuronidase; reduced glutathione (GSH); and aflatoxin-GSH conjugate. Colon mucosa was obtained by opening the colon, gently flooding away intestinal contents with buffered saline, and scraping mucosa from the gut wall with the edge of a clean glass histology slide. The collected mucosa was then chilled on ice or frozen until assays were conducted.

The concentrations of microsomal protein in rat liver or colon mucosa did not differ between the two dietary groups per unit of liver (Table 8) but AFB_1 treatment significantly increased the microsomal protein content both in liver and in colon mucosa, a result of the enzyme-inducing capacity of AFB_1.

Microsomal BPOH activity in liver was not significantly different between control and vitamin A-deprived rats. However, in both cases, AFB_1 treatment tended to increase the activity of this enzyme. Liver PNA activity per g liver increased about the same in both groups, as a result of exposure to AFB_1; per entire liver, the supplemented group increased more than the deprived group.

3667

BPOH activity in colon mucosa (Table 9) decreased slightly in deprived rats and increased slightly in control rats when AFB_1

was administered; however, this difference was not highly significant ($p = 0.05$). PNA activity of colon mucosa decreased significantly when AFB_1 was given to both control and deprived rats.

Vitamin A deficiency increased B glucuronidase activity in the liver and in the small intestinal mucosa of both groups but decreased B-glucuronidase activity in the colon contents of the vitamin-A-deficient rats. When AFB_1 was given to deprived rats, mucosal enzyme activity was maintained in the small intestine and colon, but was decreased in the gut contents at these two gastrointestinal tract levels. Liver enzyme activity decreased in the deprived rats but increased in control rats when the two groups were given AFB_1.

The concentration of reduced GSH decreased in untreated vitamin A-deficient liver and was further decreased by exposure to AFB_1 (Table 10). Aflatoxin also reduced the concentration of the enzyme in control, vitamin A-supplemented livers. However, deprived groups had more GSH in the colon mucosa and small intestine than did vitamin-A-supplemented rats. It is interesting that AFB_1 treatment tended to increase GSH in colon mucosa in both groups of rats, but decreased GSH in small intestinal mucosa of both groups. This may be related in some way to the modified susceptibility of the colon mucosa to neopalsia.

Results of studies on aflatoxin-GSH conjugates from rats pretreated with phenobarbital and fed either vitamin-A-deficient

or vitamin A-supplemented diets are shown in table 11. The aflatoxin-GSH conjugate levels in the livers of vitamin A- deficient rats were 3-fold higher than those in control animals, but there was no difference in the concentration of conjugate in the colon mucosa.

Binding studies (DNA-AFB_1 adducts) were done in the following manner. With the onset of deficiency symptoms and decreased serum vitamin A concentrations, groups of vitamin-A-deficient and normal rats was given ^3H-AFB_1 orally. They were then sacrificed by cervical dislocation at periods ranging from 2 to 24 hours after dosing. Colon mucosa (100 mg) was obtained from each animal by carefully scraping the colon epithelial cells with a glass microscope slide. The cells were homogenized and CsCl gradients fractionated into 0.375-ml portions with a gradient fractionator while the absorbance of the effluent stream was continuously monitored at 254 nm. The absorbance of peak fractions was determined at 260 adn 280 nm and the radioactivities in all fractions were determined by a scintillation counter.

A second experiment was conducted to determine if replenishing vitamin A would modify binding characteristics. After the normal vitamin A-depletion period, a different group of deprived rats was given retinyl acetate (150 ug/rat/day) (Hoffman-LaRoche, Nutley, NJ) for 2 days. A dose of 150 uCi of ^3H-AFB_1 was given to each of these rats 24 hours after the

second dose of retinyl acetate. They were sacrificed by cervical dislocation 10, 12 or 15 hours after ^3H-AFB_1 dosing, and DNA binding studies were conducted on the colon mucosa.

The DNA of colon mucosa from vitamin-A-deficient rats bound more aflatoxin than that of normal rats (Table 12) with a peak binding 15 hours after a single oral dose of ^3H-AFB_1. Retinyl acetate supplementation significantly decreased DNA binding of aflatoxin; between supplemented and non-supplemented rats, there were 2- to 4-fold differences in DNA binding at 12 and 15 hours. At 10 hours DNA binding in supplemented rats increased slightly, but not significantly. The deprived rats had more DNA/g of colon mucosa than supplemented rats (ug DNA/g 136 $\pm$ 243 vs 1189 $\pm$ 195 for deprived and supplemented, respectively). These observations indicate that vitamin A supplementation to deprived rats for as short a period as 48 hours reduced AFB_1 binding to a level comparable to that of control rats.

Although AFB_1 is known primarily as a hepatocarcinogen, as noted above it also induces colon cancer in rats fed diets deficient in vitamin A. In previous studies, the increased incidence of colon tumors was usually accompanied by a decreased incidence of liver tumors (Newberne and Rogers, 1976). Although the data from the present study do not clarify the mechanisms of colon tumor induction, it is possible that the observed decrease in microsomal enzyme activity in the colon or the liver of vitamin A-deficient animals resulted in a different set of AFB_1 metabo-

lites or in the distribution and binding of the proximate carcinogen.

Liver and colon BPOH activity varied little between deficient and supplemented groups; therefore, hydroxylated derivatives of aflatoxin (aflatoxin B_{2a}, aflatoxin M_1, aflatoxicol, and aflatoxin Q) are probably present in both dietary groups. In contrast, liver PNA activity was increased by AFB_1 in both groups, which could indicate increased production of the metabolite aflatoxin P_1; this assumption, of course, requires experimental validation. AFB_1 can also be metabolized by the mixed function oxidases to AFB_1-2,3-oxide, which interacts covalently with cellular macromolecules and thus alter normal biochemical processes. Results from the experiments reported here have clearly established that colon DNA in vitamin-A-deficient rats binds more aflatoxin than does DNA from normal, supplemented rats, and that this binding can be returned to levels equivalent to those of controls by supplementation with vitamin A for as short a period as 48 hours.

There was less GSH in livers of vitamn A-deficient rats, than in control rats; this was accompanied by more aflatoxin-GSH conjugate in the livers of the deficient animals, accounting in part for the decreased unconjugated GSH. The decreased GSH would not permit normal levels of other GSH conjugates and in this manner may have contributed to the greater toxicity of AFB_1 in deficient rats.

The increased incidence of colon tumors induced by AFB_1 in vitamin A-deficient rats would appear to be related to the role of vitamin A in the metabolism of the carcinogen. Vitamin A affects the binding of AFB_1 to cellular macromolecules, especially DNA. The mechanism, whether caused by the rate of aflatoxin adduct removal, by DNA repair, or otherwise, is not clear.

The ability of modified DNA to repair itself is a factor that must be considered in attempts to interpret our results. Repair of DNA in human cells after AFB_1 exposure has been reported; furthermore, AFB_1 exposure may affect the rate of removal or repair of DNA. Therefore, vitamin A may reduce carcinogenic potential by altering metabolism, which affects the binding of the carcinogen to cellular macromolecules, and thus influence the induction of colon cancer (and liver cancer) in rats. These observations suggest an important modifying role for vitamin A in experimental neoplasia and could have significant implications for human cancer in populations consuming inadequate amounts of vitamin A and diets contaminated with AFB_1. These conditions are present in many areas of the world where malnutrition, aflatoxin, and liver and colon cancer co-exist.

C) Other Nutritional Factors

(1) Selenium

Other nutritional factors influence the manner by which mammalian systems respond to aflatoxin. A single oral dose of afla-

toxin B_1 given to rats at 7 mg/kg bodyweight was less toxic in animals fed a high selenium (selenite) diet containing selenium at 1 mg/kg (2-week mortality 7/28) than in animals fed diets adequate or marginal in selenium, containing selenium (selenite) at 0.1 or 0.03 mg/kg feed, respectively (2-week mortalities 20/20 and 28/29). However, a further increase in selenium intake (5 mg/kg feed) reaching toxic levels predisposed the liver to aflatoxin injury and together with aflatoxin exposure resulted in kidney lesions (tubular necrosis at the cortico-medullary junction) (Newberne & Conner, 1974). When the level of aflatoxin exposure was 50 micrograms (10 micrograms daily for 5 days) in chronic studies of 12 months, selenium, as selenite, at concentrations of 2 to 5 ppm enhanced liver injury and the incidence of hepatocellular carcinoma (table 13).

The incidence of liver tumours in Sprague-Dawley rats given a total of 500 ug of aflatoxin B_1 intragastrically, over a 4-week period, was not influenced appreciably by dietary selenium (as selenite) contents ranging from 0.03 to 5.0 mg/kg (Grant et al., 1977) but later studies indicated that 1.0 or 2.0 ppm (ten and twenty fold the amount considered adequate) had a protective effect. These results will be published elsewhere but table 14 lists some of the results.

(2) Dietary Fat

3667

Dietary fat influences the response of rat liver to aflatoxin (Newberne, Weigert & Kula, 1979). Corn oil, compared to

beef fat resulted in a significant enhancement of liver tumors (table 15). The increased incidence of liver tumors associated with corn oil coincided with increased liver microsomal enzyme activity.

Interactions of a Nitrosamine With Aflatoxin B_1

While the occurrence of nitrates in many vegetables and plant foods has been recognized for decades (National Research Council, 1981) and acute effects on man and animals established, only recently have nitrates been scrutinized because of potential for conversion to nitrites with nitrosation of dietary amines and formation of nitrosamines. Nitrates are converted to nitrites in the gastrointestinal tract of man and animals and thus may participate in nitrosation reactions to form nitrosamines, many of which are carcinogenic to animals (Lintas et al, 1982; Searle, 1976). Furthermore, large amounts of nitrites are produced from a variety of nitrogen sources in human bodies (Tannenbaum et al., 1979). Thus, we cannot prevent exposure to nitrites and, thereore to some nitrosamines. The field of studies relative to the potential for interactions between mycotoxins and nitrosamines is virtually untouched.

Recent work has shown that nitrites alone added to the diet of rats at various levels of concentration increase the incidence of lymphoreticular tumors (Shank and Newberne, 1976). A second large study has confirmed these observations (Newberne, 1979).

These data are the first to indicate that nitrite alone may act as a carcingen, a co-carcinogen or as a promoter, in addition to participating in the formation of nitrosamines.

While we have detected variable amounts of nitrosamines in some samples of natural product rodent diets, we believe that it is premature to assign a level of significance to such contamination. Currently, there is no available evidence that such low concentrations of nitrosamines cause problems in animal experimentation, alone or in concert with other dietary factors. However, results of some studies recently completed in our laboratories (table 16) indicate that diethylnitrosamine (DEN) must reach 10 ppm in the diet before it influences a response to AFB_1 (Newberne and Conner, 1980). On the other hand, Angsubhakorn et al. (1981) have described experiments in which AFB_1 was followed by dimethylnitrosamine (DMN); this regimen resulted in more liver neoplasia than that in rats in which DMN was followed by AFB_1. In either case however there was a synergestic effect when the two carcinogens were used, compared to single exposures.

Clearly, nitrosamines are not as potent as AFB_1, but effects of combinations of various nitrosamines and other environmental contaminants are unknown and should receive the highest priority for research. Until further research answers questions about low levels of nitrosamines in feed, we should be aware of contamination but not assign it an inordinate amount of concern. If

3667

low levels of nitrosamines play a significant role in spontaneous tumors or in controlled studies, some evidence should have become apparent by now. We therefore advise caution in interpretation of results of surveys or of reports (Edwards and Fox, 1979) that suggest that low levels of nitrosamines in experimental animal feeds warrant discarding valuable data obtained at great cost and effort. These suggestions are at least premature.

Exposure to Artificial Sunlight, Riboflavin and Carotene

An additional instance of the interaction of diet, toxins and other environmental factors is presented in the report of Joseph-Bravo et al. (1976). Following up on observations (Newberne et al., 1974) that an interesting interaction between light energy, riboflavin and carotene modified the response of rats to aflatoxin, Joseph-Bravo went on to show that this was due in part to a difference in metabolism of aflatoxin. In the acute studies weanling male Sprague-Dawley rats were fed either a control diet or a diet marginal in lipotropes and dosed with either aflatoxin B_1 or monocrotaline. Some from each group were given an excess of either riboflavin or carotene prior to administration of the toxins and then exposed for 2 hours to artificial sunlight. The marginal lipotrope diet gave protection from the acute effects of both toxins. Exposure to light enhanced the toxicity of aflatoxin, and light plus riboflavin synergistically increased the toxicity. Carotene alone or with light protected against aflatoxin. Light protected the rats from the acute toxi-

city of monocrotaline, but riboflavin had no further significant effect. These studies indicate interactions between light, vitamins and hepatotoxins, suggesting that environmental factors may influence the potency of drugs and chemicals.

In follow-up studies (Joseph-Bravo, 1976) rats were given low levels of aflatoxin 5 days/wk for 3 wks; 30 min after each dosing, half of them were irradiated for 2 hr. In some, levels of glucose-6-phosphatase and acid phosphatase were determined 5 days after completion of treatment. Table 17 suggests that, although aflatoxin induces glucose-6-phosphatase, UV-irradiation decreased the enzyme induction.

Remaining rats were killed at 30 or 53 wks. All underwent complete necropsies and histopathologic examination. In the second experiment, rats were dosed with riboflavin and divided into four groups: no further treatment; aflatoxin (LD_{50}); irradiation (1-2 hr.); or aflatoxin plus irradiation. Blood riboflavin levels were determined at intervals following these treatments. Data in table 18 is significant in again illustrating that AFB_1 depresses nutrient levels in serum, and usually in tissues.

In the third experiment, the chemical reactions of irradiated aflatoxin and/or riboflavin were studied by ultraviolet spectroscopy and thin layer chromatography (TLC). The 53-wk study showed clearly that light decreased the incidence of

aflatoxin-induced cancer. The other results may provide an explanation. Aflatoxin caused blood riboflavin levels to decrease- an effect enhanced by irradiation, suggesting that photosensitized riboflavin and aflatoxin form a complex. This interpretation gains support from studies in vitro that showed that riboflavin quenched aflatoxin photodegradation, perhaps by complexing with aflatoxin. Thus, low carcinogenic doses of aflatoxin may complex with endogenous, photosensitized riboflavin, inhibiting its degradation into carcinogenic metabolites. A further consideration is that, UV energy, acting on AFB_1 circulating in superficial capillaries of the skin may influence the molecule directly resulting in a less toxic metabolite, or, conversely, enhancing toxicity by promoting the conversion of the parent compound to more toxic metabolites. In our studies the former would appear to be the case.

Cyclopropenoid fatty acids (CPFA). Cyclopropenoid fatty acids (CPFA) which occur for example in cottonseed oil, enhanced tumour induction in trout by both aflatoxin B_1 and aflatoxin M_1 (Sinnhuber et al., 1968a). Young trout were fed a purified diet which contained an aflatoxin B_1 concentration of 4 ug/kg with or without the addition of CPFA at 220 mg/kg diet. Hepatomas were found in 27/30 fish fed CPFA and necropsied after 6 months; at 9 months, 20/20 bore hepatomas. Corresponding incidences of hepatomas in fish that did not receive CPFA were 0/30 and 4/20, respectively (Sinnhuber et al., 1968b). In later

experiments, fish were fed aflatoxin M_1 at the rate of 4 ug/kg diet, with or without the addition of CPFA at 100 mg/kg. The incidences of hepatomas in CPFA-fed fish were 6/40 at 4 months and 42/63 at 12 months. Corresponding incidences in fish not fed CPFA were 2/40 and 6/40 respectively (Sinnhuber et al., 1974).

On the other hand, the enhancing effect of CPFA on aflatoxin hepatocarcinogenesis was not clearly evident in several studies on rats (Friedman & Mohr, 1968; Lee et al., 1969a; Nixon et al., 1974). No significant increase in liver tumours was observed in Wistar male (M) and female (F) rats when sources of CPFA such as food grade cottonseed oil (CSO) or Sterculia foetida oil (SFO) were added at levels of 10% and 0.04%, respectively, to diets containing aflatoxin B_1 at concentrations of 20 or 100 ug/kg. Feeding these diets for different perids (generally exceeding 500 days) at the aflatoxin level of 20 ug/kg resulted in hepatomas in 4/36 rats (M: 2/17; F: 2/19) with CSO exposure, in 1/37 rats (F:1/19) with SFO exposure, and in 0/38 rats without CSO or SFO in the diet. With an aflatoxin B_1 exposure level of 100 ug/kg diet, the incidences of hepatomas in the CSO group, SFO group, and the group withou CSO or SFO were 15/36 (M: 12/17; F: 3/19), 17/37 (M: 10/17; F: 7/18), and 15/35 (M:7/17; F: 8/18), respectively. In Fischer rats, CSO was tested only in combination with the lower concentration of aflatoxin; with an aflatoxin B_1 level of 20 ug/kg diet, the hepatoma incidence was 6/31 (M: 4/15; F: 2/16) with CSO, and 6/28 (M: 5/13; F: 1/15) without CSO exposure (Nixon et al., 1974).

Influence of Factors Other Than Diet

Goodall and Butler (1969) studied the effects of hypophysectomy on rats fed a diet containing 4 ppm aflatoxin B_1. All of the control animals developed liver tumors in 49 weeks, whereas none of the hypophysectomized animals developed tumors in the same period of time. Hypophysectomized rats, however, grew little if any over the period of the experiment, whereas the other animals grew normally. This discrepancy precluded an adequate interpretation of results, and, insofar as is known the study has not been repeated. Similar observations were also made under analogous conditions in experiments with aminofluorenes (Shirasu et al., 1966).

Newberne and Williams(1969) reported that rats fed diets containing 0.2 ppm aflatoxin B_1 and 4 ppm diethyltilbestrol developed fewer liver tumors than those that were treated with aflatoxin alone. The study included paired-feeding and feeding-to-weight, indicating that decreased tumor incidence associated with diethylstilbestrol was not the result of decreased food intake. The specific mechanisms responsible for protection remained obscure. Further experiments (Newberne et al., 1967) showed that methionine fed at 0.2% of the diet acted synergistically with aflatoxin B_1 at 0.4 ppm to induce liver cancer in rats. However, aflatoxin B_1 alone at dietary levels of 0.4 of 1.5 ppm was more effective in inducing liver tumors than in combination with urethane at 0.1-0.6% of the diet. Studies in

which rats were exposed to rubratoxin B and to aflatoxin B_1 indicated a sensitization to rubratoxin, but the evidence for potentiation of carcinogenesis was equivocal (Wogan et al., 1971).

Reddy and Svoboda (1972), studying the effect of lasiocarpine given to rats with and without aflatoxin, found that the pyrrolizidine alkaloid (lasiocarpine) was a hepatocarcinogen in itself; moreover, it modified the morphologic pattern of livers in which tumors developed. It also resulted in a postnecrotic cirrhosis which is not characteristic of aflatoxin alone. Newberne and Rogers (1973) found that another pyrrolizidine alkaloid, monocrotaline, also was carcinogenic for the rat when exposure was chronic, and that it also modified the histologic structure of livers in which tumors developed as a result of exposure to both aflatoxin and monocrotaline. As in earlier studies in which lasiocarpine failed to inhibit the induction of liver tumors by acetylaminofluorene (Rogers and Newberne, 1971), monocrotaline failed to prevent the induction of liver cell tumors by aflatoxin, despite its potent antimitotic effect (Bull et al., 1968). Monocrotaline was more potent as a syncarcinogen when the diet was low in lipotropes.

McLean and Marshall (1971) investigated the influence of phenobarbitone on the induction of liver cancer by aflatoxin B_1. The aflatoxin was fed to rats at 5 ppm in the diet for 9 weeks; during this period the experimental group which received con-

3667

tinuously phenobarbitone and aflatoxin had a lower incidence of liver tumors and a longer lag time in tumor development in comparison to rats fed aflatoxin alone. McLean and Marshall suggested that phenobarbitone induced liver microsomal enzymes that metabolized aflatoxin to a noncarcinogenic form or somehow accelerated its excretion.

The remainder of this manuscript will describe the effects on the liver of one of the major disease-producing mycotoxins, aflatoxin B_1, according to species of animal.

Turkey

Histologic changes induced in the turkey by feeds contaminated with aflatoxin have been described by Siller and Ostler (1961) and by Wannop (1961). Birds dying during the early stages of intoxication had severe periportal hepatic parenchymal cell necrosis and venous congestion. Nodular regeneration accompanied the diffuse necrosis of parenchymal cells and the concomitant biliary proliferation. There was little or no inflammatory reaction and only a small increase in collagen. Since these early observations were reported, the pathologic changes induced in turkeys by aflatoxin have received little attention. Magwood et al. (1966) described an "induced tolerance" to aflatoxin poisoning which appeared to be unrelated to the changes seen in the liver. After 23 weeks on a toxic diet, the livers were nodular with a dissecting fibrosis, biliary proliferation, and marked variation in the size of parenchymal cell nuclei.

Duckling

Hepatic lesions induced in the duckling by the aflatoxins form the basis of the bioassay system originally described by Sargeant et al. (1963). The acute LD_{50} of the aflatoxins has been estimated in a variety of solvents, and results from several different laboratories indicate general agreement for the B_1 fraction (see Table 1). Less is known about the other three fractions.

The lesion induced by a single dose of aflatoxin B_1 was described in detail by Butler (1964). A periportal zone of parenchymal cell necrosis with the formation of lakes of fat developed over a 48-hour period. Extensive biliary proliferation, reaching a maximum at three days, was associated with the parenchymal cell necrosis (Fig. 1). The livers of normal ducklings contain a large amount of lipid on the first day of life as a carryover from the yolk, but this disappears 4-5 days after hatching. In ducklings dosed with aflatoxin, there is a delay in the removal of the lipid, but there is little or no increase in fat content compared to control birds. At 14 days postexposure, there was an increase in mitotic activity of parenchymal cells of birds surviving an LD_{50} dose.

Levels representing ¼-½ the LD_{50} dose resulted in less severe lesions, and there was little correlation between dose and degree of biliary proliferation. A wide variation in response to

366

a given dose was observed among individual birds, and this contributed to the apparent lack of uniform biliary proliferation within groups. A hemorrhagic periportal necrosis was often induced with doses greater than the LD_{50}. The development of biliary proliferation was at first thought to be characteristic of and specific for aflatoxin. However, a similar lesion was described which was associated with the administration of dimethylnitrosamine and cycasin, but not with carbon tetrachloride, thioacetamide, or the alkaloids retrorsine and indecine. The observation that histologic changes similar to those observed with aflatoxin were induced by dimethylnitrosamine was confirmed by Carlton et al. (1966).

Asplin and Carnaghan (1961) described an acute disease in day-old ducklings fed toxic peanut meal. The birds failed to grow and there were macroscopic hemorrhages in many of the organs and tissues. Extensive biliary proliferation and parenchymal cell degeneration were observed in the liver: these changes were followed by cirrhosis in many of the birds. Newberne et al. (1964) have shown that the repeated administration of purified aflatoxin or continuous feeding of Aspergillus flavus extracts or peanut meal naturally contaminated with the aflatoxins produced the same pathologic lesions seen by earlier workers; they described in detail the development of the liver lesion up to four weeks. The severity of the lesion was related to the amount of aflatoxin administered. Two days after exposure, there were

parenchymal cell necrosis and early biliary proliferation; the latter progressed in parallel with increased severity of parenchymal cell necrosis. Seven days after exposure, mitotic figures were present in the parenchymal cells and biliary proliferation was still active. Widespread nodular regeneration was observed after four weeks. Madhavan and Rao (1966) reported hepatic infarcts in ducklings given 10-40 ug of aflatoxin a day for five days, but this has not been confirmed by other workers and appears to be uncommon. Carnaghan (1965) described the development of hepatic parenchymal cell tumors in ducks fed 0.035 ppm of aflatoxin, as a contaminant in peanut meal, for 14 months; Newberne (1965) observed similar tumors in ducks after 16 months exposure to feed containing contaminated peanut meal. Cirrhosis was present in all birds in the latter experiments whether or not liver cell tumors developed.

Chicken

A spontaneous disease of young chickens attributed to toxic peanut meal was reported by Asplin and Carnaghan (1961). The livers of birds were described as firm and pale in color. Histologically, degenerative and regenerative changes were seen, and, by four weeks, regenerating nodules of parenchymal cells were present. In birds fed aflatoxin-contaminated peanut meal, there was a progressive lymphoid hyperplasia; at the end of four months, there were no regenerative nodules, but large multiple focal areas of lymphoid hyperplasia were observed. Similar

changes have been described by Loizelier (1963) and by Raimo et al. (1962).

Carnaghan et al. (1966) studied the experimental poisoning of chickens with toxic peanut meal contained in a diet which assayed about 1.5 ppm of the B_1 fraction. Only one animal fed this diet died, and clinical manifestation of toxicity was limited to slower growth. During the first few weeks of the experiment, the livers of those fed the aflatoxin-contaminated diet were enlarged and pale, and some of them contained petechial hemorrhages. Later, there was a progressive nodularity of the surface. Microscopically, the first change was seen after 3.5 days and consisted of a periportal fatty infiltration; this lesion progressed for the next 3-4 weeks. Associated with the fatty change were scattered liver cell necrosis, progressive biliary proliferation, and an increase in connective tissue. After four weeks on experiment, regenerating parenchymal cells with large nuclei were arranged in ductular fashion; these lesions were seen along with individual cell necrosis. Large aggregates of polymorphonuclear leukocytes and lymphocytes were seen in the portal tracts. After six weeks, biliary proliferation, fibrosis, and lymphocytic hyperplasia of the portal tracts increased. Foci of regenerating parenchymal cells were present with some nuclear enlargement. After eight weeks exposure, when the experiment was terminated, the parenchymal cells were surrounded by areas of bile ducts and fibrous tissue containing large focal areas of lymphocytic hyperplasia.

Cattle

The first report of poisoning in cattle by Brazilian peanut (groundnut) meal was that of Loosmore and Markson (1961). Calves, 3-9 months of age, had eaten for at least six weeks a compounded food containing 15% Brazilian peanut meal (not assayed for aflatoxin content). The livers of the animals exhibited areas of fibrosis with biliary proliferation and venoocclusive disease similar to that described in ragwort (Senecio jacobea) poisoning (Markson, 1960). Clegg and Bryson (1962) reported an outbreak occurring at about the same time in cattle 1.5-2 years old, with symptoms and lesions identical to those described above; the senior author has observed similar pathólogic alterations in the liver of cattle from India.

Allcroft and Lewis (1963) investigated experimental poisoning of calves and older cattle by compounded food containing 2.0 ppm of aflatoxin. Liver biopsies were taken monthly and post-mortem examination was performed after four months exposure (Hill, 1963). Progressive biliary proliferation, an increase in connective tissue, and some degeneration of centrilobular hepatic cells were described. The livers of animals killed after 11 weeks on the diet had complete disruption of the lobular pattern and an increase of connective tissue which coursed throughout the liver lobule; many of the central veins were partially or completely obliterated by fibrous tissue (Fig. 2). Throughout the lobule, parenchymal cells were isolated by strands

of connective tissue. Structures resembling small bile ducts were scattered throughout the lobule, and there was a mild necrosis and pleomorphism of parenchymal cells (Fig. 3) located away from the periportal area, but mitotic figures were not seen in either the parenchymal or biliary cells of the material examined.

Pig

Weanling pigs, 6-7 kg, were used to determine acute effects of aflatoxin B_1 on this species (Newberne, 1967). Oral administration indicated an LD_{50} of 0.62 mg/kg, and doses of 1.0 to 2.0 mg/kg resulted in acute death in 18-24 hours. Lower doses permitted some of the animals to survive and those alive after seven days were sacrificed.

The principal lesions were similar to those seen in other species, namely, liver damage and hemorrhage. The liver was swollen, congested, and friable; occasional petechiae were visible on the liver surface, and animals surviving beyond 24 hours often had ascites and hydrothorax. The gall bladder was edematous, and the mucosa was petechiated and ecchymotic, similar to that observed in dogs. Microscopically, centrilobular necrosis with a mild fatty change and some hemorrhage was seen (Fig. 4). In subacute cases, parenchymal cell necrosis was less pronounced, but the normal lobular appearance of the liver was accentuated by biliary proliferation.

Field cases of the natural disease (Loosmore et al., 1961) and cases of experimental disease induced by feeding contaminated

meal (Cuthbertson et al., 1967) were the same as those induced by multiple administration of mixtures of toxins (Wilson, 1967). Distortion of the lobular pattern of the liver by dissecting fibrosis and biliary proliferation was seen microscopically, and there were scattered areas of degenerative changes, variation in nuclear size, and nodule formation in all livers examined (Fig. 5).

Sheep

Sheep appear to be the species most resistant to aflatoxin, with no field cases reported thus far. Abrams (1965) reported that sheep were not susceptible to low levels of aflatoxin but were sensitive to doses of 3-4 mg twice weekly for 4-6 weeks; however, details of pathologic changes, if any, were not described. Lewis et al. (1967) reported long-term feeding trials using contaminated meal (1.75 ppm of aflatoxin in the diet) which resulted in one hepatic carcinoma at 3.5 years and two nasal tumors at four and five years.

Rats

Following the recognition of aflatoxin poisoning among farm animals, the rat has been used extensively to study the acute toxicity and carcinogenicity of the aflatoxins. Particular emphasis has been placed on acute toxicity of the B_1 fraction of the aflatoxin complex. The youngest animals are most susceptible

3667

(see Table 2), with sex and route of administration affecting the response. In most experiments the rats usually died between three and seven days after exposure (Burnside, 1957); mature females were considerably more resistant, a characteristic which appears to be lost during the latter part of pregnancy (Butler, 1966).

Lesions induced by an LD_{50} dose of aflatoxin B_1 include a periportal zone of necrosis (Butler, 1964) which develops during a three-day period after dosing. This is accompanied by a marked biliary proliferation (Fig. 6). The necrotic debris is removed by macrophages, but rapid regeneration of parenchymal cells comparable to that which follows either partial hepatectomy (Abercrombie, 1951) or toxic injury due to carbon tetrachloride is not seen. At three days postexposure, only occasional mitoses are seen in parenchymal cells, although there is active mitosis of the biliary cells, and slow recovery continues for many weeks. The delay in mitotic activity of the parenchymal cells was studied by Rogers and Newberne (Rogers, 1967), who demonstrated a two-day inhibition of mitosis following a dose of 3 mg/kg which also resulted in a scattered individual cell necrosis.

Two weeks after a single LD_{50} dose of aflatoxin B_1, prominent biliary proliferation persisted along with mild mitotic activity of parenchymal cells, but the striking feature was the development of enlarged hyperchromatic nuclei. One month after a single dose, this lesion was often as marked as that seen after

continuous administration. Biliary and oval cell proliferation of a magnitude that distorted the normal lobular pattern was seen in some animals, and many of the parenchymal cells had large bizarre nuclei (Fig. 7), some of which were located in an occasional small regenerative nodule. The development of the lesion following a single dose has not been studied further in any detail but by 18 months the survivors showed a slight residual irregularity of parenchymal nuclear size and a minimal residual biliary proliferation, but hepatic tumors were not seen. Seven of 15 female rats surviving a dose of 7.0 mg/kg B_1 developed hepatocellular carcinoma after two years (Carnaghan, 1967).

Although the nonpregnant, mature female is less susceptible to the acute effects of the toxin, the lesion when induced is similar to that in the male. A periportal zone of necrosis develops along with biliary proliferation. The main difference is the greater accumulation of fat in the female than in the male. In neither sex is there hemorrhagic necrosis in the liver.

Prior to the isolation of aflatoxin, Lancaster et al. (1961) showed that a diet containing meal toxic to poultry also induced hepatic carcinomas in rats. Salmon and Newberne (1963) reported a high incidence of hepatomas in rats fed a diet containing peanut meal as a source of protein, and the development of the hepatic lesion induced by feeding contaminated meals assayed for aflatoxin was described by Butler and Barnes (1963). At high dietary levels (4-5 ppm of aflatoxin B_1) the lesion induced was

3667

similar to that produced by other carcinogens, including ethionine, dimethylnitrosamine, and 4-dimethylaminoazobenzene. The earliest change was seen at 3-4 weeks; it consisted of a biliary and oval cell proliferation with an increasing irregularity in the size of the parenchymal cell nuclei. This lesion progressed and, by 9-12 weeks, there was marked biliary proliferation and many large hyperchromatic parenchymal cell nuclei (Fig. 8). Scattered foci of parenchymal cells with small uniform nuclei and deeply basophilic cytoplasm were also observed. Small ill-defined regenerative nodules were also seen, but at no stage was there a marked increase in fibrous tissue. Cholangiofibrosis was only rarely seen. The first hepatic carcinomas seen at 35 weeks were similar to those described for other liver carcinogens (Butler et al., 1963; Newberne, 1964), but the diagnosis of a cholangiocarcinoma could be made in only one case.

The incidence of tumors was 100% when peanut meal containing 4-5 ppm of aflatoxin was fed. Aflatoxin levels as low as 0.7-0.8 ppm resulted in an incidence of 100% also, but there was a longer latent period (up to 82 weeks). At these low levels, the early lesions were much less obvious and were seen only after many weeks on the diet. Lesions included mild oval cell proliferation and a few parenchymal cells with enlarged nuclei. At a later stage, when carcinomas were observed, there was no evidence of cirrhosis (Fig. 9).

When purified aflatoxin became available, it was confirmed that the carcinogenic action of the peanut meal was a result of

contamination with aflatoxin (Barnes, 1964). Subsequent investigations have attempted to establish the lowest dose level and minimal exposure time required to induce hepatic carcinomas. Using purified aflatoxin B_1, Wogan and Newberne (1967) have shown that levels as low as 0.015 ppm in continuous feeding results in 100% incidence of hepatic carcinomas. The intubation of 0.4 mg of aflatoxin B_1 over a period of 14 days resulted in a lower incidence of carcinomas (17%) and a longer latent period (up to 82 weeks). A summary of the feeding experiments reported by Wogan and Newberne is given in Table 3. There is an apparent difference in susceptibility in the two groups of experiments referred to above. The feeding trials using contaminated meal at low dose levels showed a sex difference. Moreover, there was a reduced incidence in the males exposed to levels of aflatoxin as a feed contaminant compared to those administered purified aflatoxin which was added to the diet. These observations represent a number of variables inherent in using different strains of animals, in chemical assay versus gravimetric measurement of dosing, and others. The meal-feeding experiments used Wistar/Porton rats while the purified aflatoxin studies used Fischer-strain animals. The aflatoxin content of the former was chemically assayed while the latter used crystalline aflatoxin B_1 administered in doses measured gravimetrically.

The sequential development of the lesions (induced by purified aflatoxin B_1) similar to those described for the con-

taminated meal (Butler et al., 1963) are described in detail by Newberne and Wogan (1968). The carcinomas were typical hepatocellular carcinomas, many of which had metastasized to the lungs. Liver cirrhosis was not observed in any of the animals, a point of some importance since many chemical carcinogens are associated with cirrhosis. The comparative carcinogenic activity of the various aflatoxins is not known at present.

Guinea Pig

Paterson et al. (1962) reported the experimental induction of a disease in guinea pigs similar to a natural outbreak described by Paget more than a decade earlier (1954). The disease was referred to as "exudative hepatitis," an erroneous term, since the lesions described were not those associated with an inflammatory process. Histologically, the livers showed a marked dilatation of the periportal lymphatics termed "tubular dilatation of the liver cell columns." Little parenchymal cell necrosis was seen, and biliary proliferation was not reported. The experimental induction of the disease by feeding a diet containing 15% peanut meal indicates that the syndrome was related to aflatoxin contained in the diet. Similar lesions have been reported by Clegg and Bryson (1962) and further strengthen the case for aflatoxin contamination of the feed in earlier disease outbreaks in guinea pig colonies.

Experimentally, the acute effects of single doses of aflatoxin B_1 in guinea pigs have been studied by Butler (1966). The

LD_{50} is 1.4 mg/kg body weight with 95% confidence limits of 1.05-1.8 mg/kg. No significant sex difference was observed. The LD_{50} dose induced a centrilobular necrosis after 24 hours which progressively increased in severity to 72 hours (Fig. 10). Associated with parenchymal cell necrosis was a periportal fatty change. The portal tracts were normal after 24 hours except for dilatation of the lymphatics similar to that described in the feeding experiments. After 48 hours a few mitoses were seen in the small bile ducts but not in the parenchymal cells. At 72 hours the mitotic activity of bile duct epithelium was prominent, and there was proliferation of small ducts. Centrilobular necrosis was well-developed at this point, but the periportal fatty change had decreased, and mitosis in the parenchymal cells had subsided.

Necrotic areas were replaced after four days by macrophages, but there was very little evidence of parenchymal cell regeneration. Biliary proliferation was pronounced at this stage, with ductal cells radiating out from the portal area to separate parenchymal cells into groups or as single units. At seven days postexposure, a few macrophages were observed in the centrilobular zone, but most of the lobule consisted of normal parenchymal cells with an occasional mitotic figure. At the periphery of the lobule, many of the parenchymal cells were isolated by the continued biliary proliferation (Fig. 11); some of the parenchymal cells contained lipid while others were undergoing lysis. By ten

days the biliary proliferation was still marked, but mitotic figures were not seen and there was a normal lobular pattern with no residual evidence of necrosis. Parenchymal cells were normal and only an occasional mitotic figure was observed. Animals studied three months after exposure had normal livers, but a few animals appeared to have a slight increase in the connective tissue component located about the main portal tracts with a small residual component of proliferated bile ducts remaining among normal parenchymal cells.

The toxic effects of groundnut meal assayed for aflatoxin were reported by Butler and Barnes (1963). Animals developed ascites and edema; this had been described by previous workers. At a level of approximately 1.5 ppm of aflatoxin in the diet, survival was between two and four weeks. The liver contained varying degrees of biliary proliferation which extended into the lobules, and the periportal lymphatics were dilated (Fig. 12). In the periportal zone, the fat content of the parenchymal cells was increased and necrotic parenchymal cells were scattered throughout the lobule. At a dietary level of 0.7-0.8 ppm of aflatoxin, the animals lived for up to eight weeks. Biliary proliferation was observed in the livers of three animals, but it was not as prominent as that observed at the higher dose levels. Scattered throughout the lobules were areas of parenchymal cells undergoing lysis and a concomitant tubule formation (Fig. 13). The lowest dose level reported was 0.35-0.4 ppm of aflatoxin; at

this level most animals were dead by 27 weeks with only one survivor to 44 weeks. During the course of the feedings, there was a progressive biliary proliferation and individual cell necrosis similar to that previously described. By the 28th week following initiation of the experiment, islands of parenchymal cells appeared as regenerating nodules in which a few pyknotic nuclei and an occasional mitosis were seen. The solitary survivor at 44 weeks had a coarsely nodular liver containing broad bands of fibrous tissue, collections of bile ducts, and some regenerative nodules with bizarre parenchymal cells.

In order to obtain longer survival times, the dose was further reduced to 0.15 ppm of aflatoxin in the diet. This dose level resulted in a high mortality, but six animals survived an average of 106 weeks. The longest survival time was 160 weeks. One of these animals, killed at 127 weeks, had an anaplastic hepatocarcinoma, the only hepatic tumor seen in guinea pigs treated with diets containing aflatoxin (Fig. 14).

Mouse

There is no detailed account of the acute lesions induced by aflatoxin in mice. The LD_{50} has been estimated as about 9 mg/kg. However, a problem with solvent toxicity makes this figure unreliable. In feeding trials mice appear to be resistant to the chronic toxicity of aflatoxin. Plantonow (1964) described three-month feeding trials which failed to produce any change.

3657

Newberne (1965) fed male white Swiss mice for 16 months on a peanut meal diet contaminated with 1.0 ppm of aflatoxin, and 15% of the mice developed liver tumors; two other strains of black mice fed a diet containing 1.0 ppm of purified aflatoxin B_1 failed to develop significant liver lesions.

The livers of mice that were fed contaminated peanut meal developed liver cell tumors and widespread pleomorphism of the nontumorous liver cell nuclei. Large numbers of the tumor cells contained globular, eosinophilic structures in the cytoplasm which were periodic acid-Schiff positive (Fig. 15). At the junction of normal and neoplastic cells, mitotic figures were occasionally seen. None of the tumors observed in the livers of mice were large and none had metastasized; whether these neoplasms were malignant is open to debate.

Dog

Newberne et al. (1966) have studied the effects on dogs of single and repeated doses of aflatoxin. The LD_{50} is about 0.5-1 mg/kg, with the earliest histologic change appearing as an increase in fat with congestion of the centrilobular zone and parenchymal cell necrosis (Fig. 16). At seven days there was a prominent biliary proliferation. Although this review does not consider organs other than the liver, the edema and hemorrhage seen in the gall bladder are such that they warrant brief attention. The wall of the gall bladder in dogs exposed to aflatoxin

was greatly thickened and microscopic examination revealed severe subserosal and submucosal edema and hemorrhage (Fig. 17).

Experimental studies and observations of field outbreaks have shown that the dog is very sensitive to the acute effects of aflatoxin (Newberne, 1966; Wilson, 1967). Lesions observed in spontaneous outbreaks of canine toxicosis where aflatoxin has been isolated from feed samples have been remarkably similar to those seen in the experimentally induced disease using crude or purified aflatoxin. Lesions induced experimentally were similar to those reported for "hepatitis X" (Newberne, 1955), a toxic disease of kennel dogs of the southeastern United States, reported in 1955. At necropsy the animals fed commercial dog feed containing toxic peanut meal were jaundiced, with swelling and yellowish discoloration of the liver and edema of the gall bladder identical to that seen with crude or purified aflatoxin. Histologically, fatty change with centrilobular parenchymal necrosis and biliary proliferation were sen similar to the acute single-dose lesions induced experimentally.

Cat

Adult mixed-breed cats exhibited a sensitivity to purified aflatoxin B_1 similar to the rabbit, dog, and guinea pig with a single dose LD_{50} of 0.55 mg/kg. Most acute deaths occurred in 18-72 hours; grossly the liver was swollen, pale, and friable with occasional petechial hemorrhages. Microscopically, the

3667

immediate periportal zone was pale because of decreased staining of parenchymal cells, and there was very little lipid accumulation (Fig. 18). Animals sacrificed in terminal stages had pale liver cell nuclei with a "washed out" appearance and little stainable chromatin; glycogen accounted for part of the observed nuclear change (Fig. 19). There was a mixed leukocytic infiltration in the periportal zones, and bile duct hyperplasia was observed in the livers of cats surviving beyond 72 hours.

Rabbit

The rabbit appears to be even more sensitive to the acute effects of aflatoxin than other mammalian species (Table 2). The LD_{50} for Dutch belted males and females was about 0.3 mg/kg with no significant difference between sexes or route of administration (i.p. or p.o.). Microscopically, there were hemorrhage and parenchymal cell necrosis in the midzone areas of the lobule (Fig. 20), and scattered single-cell necrosis was seen in the centrilobular area. Animals surviving the immediate lethal effects of the single dose of aflatoxin developed the mild-to-moderate bile duct hyperplasia seen in other species.

Feeding experiments have not been reported in the rabbit.

Monkey

Monkeys have been shown to be susceptible to the acute toxicity of both purified aflatoxin and contaminated peanut meal.

Doses of 500 ug for 18 days followed by doses of 1 mg/day to rhesus monkeys resulted in deaths at 32 and 34 days. At higher dose levels, deaths occurred earlier. Histologically, the livers showed fatty infiltration, biliary proliferation, and portal fibrosis (Madhavan, 1965). Cuthbertson et al. (1967) studied the effects of contaminated peanut meal on cynomologus monkeys and described liver cell damage and biliary proliferation at dietary levels of 5 ppm of aflatoxin. At lower dietary levels (1.8 ppm of aflatoxin), animals survived three years. One animal had a coarse nodular cirrhosis, while the other monkey exhibited irregular size of parenchymal cell nuclei (Fig. 21).

Other Species

Although single dose experiments have not been reported, Allcroft and Lancaster (personal communication, manuscript in preparation) have demonstrated that ferrets are extremely sensitive to toxic peanut meal diets. A diet containing 20% of a toxic peanut meal resulted in the development of liver tumors in five of seven male ferrets after 24-37 months. Decreasing the dietary level of the toxic meal to 3% resulted in 100% incidence of liver tumors.

The acute LD_{50} of aflatoxin B_1 for hamsters has been reported as 10.2 mg/kg (Wogan, 1966), but no information is available as to the pathologic changes induced. Results of preliminary feeding experiments reported by Chesterman and Pomerance

(1965) were inconclusive. At a dietary level of 2 ppm of aflatoxin, the animals failed to grow and died after 8 weeks. However, both the dosed and control animals showed the same well-developed cirrhosis with regenerative nodules, fibrosis, and biliary proliferation. The etiology of this lesion is not understood and implies other complicating factors.

The acute and chronic toxic effects in trout have been investigated by Halver (1965) following a high incidence of hepatic tumors in hatchery-raised trout in 1960. The acute LD_{50} of combined aflatoxin B_1 and G_1 has been estimated at between 0.5 and 1.0 mg/kg; the principal lesion described was a hemorrhagic necrosis. Feeding experiments resulted in biliary proliferation, with some cyst formation after six months. Associated with this was a nodular proliferation of parenchymal cells (Fig. 22) and subsequent development of parenchymal cell carcinoma.

DISCUSSION

The first significant reports of what appears to have been aflatoxin poisoning in domestic animals were those of Newberne et al. (1955) and Burnside et al. (1957). The disease in dogs was referred to as "hepatitis X" and was traced to the diet which contained peanut meal (Newberne et al.). Although the disease was reproduced by feeding the toxic feed, the exact nature of the etiologic agent was not revealed. During the same period, Burnside et al. isolated toxin-producing strains of Aspergillus

flavus and Penicillium rubrum from an outbreak of toxicosis in swine fed moldly corn. Emphasis at the time was placed on the P. rubrum culture, while studies of A. flavus were not pursued further. Le Breton et al. (1962) mention a high incidence of liver tumors in a colony of rats in Morocco fed a diet containing peanut meal. It has been pointed out (Newberne, 1955) that "hepatitis X" has now been shown to be related, at least in part, to aflatoxin-contaminated peanut meals in the diet. Paget (1954) has described a disease of rather sporadic incidence in guinea pigs which has been shown to be similar to that produced by peanut meals known to be contaminated by aflatoxin. Schoental (1961) showed that the Medical Research Council (MRC) guinea pig diet would induce hepatic carcinoma in rats. Thus, it appears that aflatoxin has a much longer history than current research work indicates.

Since the aflatoxins were first isolated (Sargeant, 1961), there has been considerable progress in the investigation of the carcinogenicity and acute toxicity from both the structural and biochemical aspects. The structures have been elucidated (Asao, 1965) and racemic mixtures synthesized (Buchi, 1966). The acute lesion in the rat has been investigated by many workers; its striking features are the periportal distribution of parenchymal cell necrosis, bile duct proliferation, slow recovery, and, in some cases, eventual development of hepatic carcinoma. It has been suggested that the carcinogenic action of aflatoxin B_1 in

the rat results from a capacity to bind to DNA, a characteristic similar to that of actinomycin D (Clifford, 1967; Sporn, 1966). However, lethal doses of actinomycin D do not produce hepatic parenchymal cell necrosis. In all species studied, the organ most affected is the liver, although other organs, particularly the kidney, show signs of damage. The distribution of the hepatic lesion is not consistent from species to species, i.e., rat and duckling, periportal; guinea pig and swine, centrilobular; dog, periportal and centrilobular; and rabbit, mid-zonal. In contrast, most other hepatotoxins, such as carbon tetrachloride, regularly induce a centrilobular lesion in both rats and guinea pigs.

There is a wide range in the acute LD_{50} dose of aflatoxin B_1, varying from 0.3 mg/kg for ducklings to 16 mg/kg for mature female rats (Table 2). In species for which data are available, the young appear to be more susceptible than mature animals.

The most striking and important feature of the investigations described in this review is the carcinogenic action of the aflatoxins in fish, birds, and mammals. When one considers that doses of 0.015 ppm of B_1 in continuous feeding or a total of 0.4 mg over 14 days resulted in a high incidence of hepatic carcinoma, and when these doses are compared with other hepatocarcinogens, it becomes clear that aflatoxin is the most potent liver carcinogen so far recognized. One further interesting result has been the demonstration that choline deficiency itself

does not appear to be sufficient to induce hepatic carcinoma (Newberne, 1966), nor is cirrhosis a prerequisite or concomitant of aflatoxin carcinogenesis. Choline deficiency can induce a cirrhotic liver in the rat, but in recent experiments carcinoma did not result unless aflatoxin or some other hepatocarcinogen was included in the diet (Newberne, 1964). Even at high doses, aflatoxin alone seldom induced cirrhosis in the rat. In most of the tumor-bearing animals, the carcinomas arise in liver with otherwise normal lobular patterns.

The recognition of the possible hazard to humans consuming contaminated foods has stimulated many programs of investigation in those areas with a high incidence of hepatic carcinoma. These have taken two main forms: (a) epidemiologic studies to compare the pattern of disease in high and low incidence areas, and (b) surveys of food for aflatoxin content. It has been shown (Loizelier, 1963; Schoental, 1961) that the toxin-producing strains of the fungus will grow on substrates which are being used as protein supplements for children, but at present there is no direct evidence that man is susceptible to the aflatoxins. The broad spectrum of animals that are susceptible makes it reasonable to conclude that aflatoxin is a potential hazard to man. However, the assumption that man responds to aflatoxin exposure in a manner similar or identical to that observed in animals is made on very tenuous grounds. We recognize that the association of liver disease, including carcinoma, with potential

3667

aflatoxin exposure in certain population groups is highly suggestive, and it provides an attractive hypothesis for use in attempting to explain the variation in the incidence of liver cancer. Reliable estimates of human exposure to the aflatoxins are not presently available; such information should derive from properly designed epidemiologic surveys, some of which are now in progress in areas where the ingestion of aflatoxin-contaminated foodstuffs may later be correlated with liver carcinoma in the indigenous population. For the present, however, we must await factual evidence and proceed with caution in assigning the aflatoxins a role in worldwide liver carcinoma.

The epidemiologic characteristics of primary liver carcinoma in Africa have been described in at least two reports (Oettle, 1964; Steiner, 1961). There are wide variations within general areas of high incidence, with the highest rate or primary hepatocellular carcinoma reported in black male Africans, particularly those from Mozambique Bantu tribes (Higginson, 1963). It is interesting that the incidence in the Mozambique group is about 500 times that reported in the U.S. population for a comparable age group (25-34 years) and 15 times that observed in natives in nearby Johannesburg.

In India there is a highly variable incidence of liver cancer (Paymaster, 1964), with a decrease in the northern and western areas of the country. There are also indications for an increase in liver cancer in the male population of Southeast

Asia. Observations in Thailand, the Philippines and Malaya (Marsden, 1958), Indonesia (Bonne, 1935), South China (Yeh, 1954), Hong Kong (where about 30% of autopsies are reported to have liver carcinoma) (W.C. Chan, personal communication), and Singapore (Shanmugaratnam, 1956) serve to reinforce the feeling among pathologists and epidemiologists that there is a trend toward increased liver cancer in many areas of the world. In Singapore the highest incidence of liver carcinoma is found in individuals who come from South China, who may have been exposed to environmental hazards in the early years of their lives.

The facts that aflatoxin-producing strains of molds are found in so many areas of the world and, furthermore, that climatic conditions favor the growth of molds in many areas where liver carcinoma occurs in a high incidence lend support to the hypothesis that the aflatoxins are involved in the etiolgy of primary liver cancer. We have pointed out previously (Newberne, 1964; 1965) that feed grade samples of peanut meal purchased on the open market have been highly contaminated with aflatoxin (1.0-5.0 ppm). Even more significant is the extremely important report by Wogan (1968) that aflatoxins have been detected at biologically significant levels in food samples collected from many parts of the world, particularly Africa and Asia. Table 4 lists food products found to contain significant amounts of aflatoxin and confirms that most major food commodities are subject to aflatoxin contamination. Although specific levels of afla-

3667

toxin content were not reported, clearly the potential hazards of the aflatoxins are evident, and they must be accorded proper attention in attempts to elucidate etiologic factors related to primary liver cancer in many population groups around the world.

CONCLUSION

It seems clear that the mycotoxins are variable but generally highly toxic; some are also carcinogenic to some animal species and to human populations. The convincing data relative to disease and to mycotoxin exposure, and in particular, aflatoxin, to a triad of problems in man (mycotoxins, malnutrition, and infectious disease) leave little doubt that mycotoxins are a worldwide problem. The fact that the toxins occur in areas of the world where malnutrition *per se* is a special problem make this a subject of particular importance to public health authorities.

References

Abercrombie, M., and Harkness, R.D. (1951) The growth of cell populations and the properties in tissue culture of segments of liver of the rat. Proc. Roy. Soc. Ser. B. 138:544.

Abrams, L. (1965) J.S. Afr. Vet. Med. Assoc. 36:5.

Adye, J., and Mateles, R.I. (1964) Incorporation of labelled compounds into aflatoxins. Biochim. Biophys. Acta. 86:418.

Allcroft, R., and Carnaghan, R.B.A. (1963) Groundnut toxicity: an examination for toxin in human food products from animals fed toxic groundnut meal. Vet. Rec. 75:259.

Allcroft, R., and Lewis, G. (1963) Groundnut toxicity in cattle. Experimental poisoning of calves and a report on clinical effects in older cattle. Vet. Rec. 75:487.

Anguidine (1971) Preclinical toxicological evaluation in dogs and monkeys. Contract NIH-71-2264, NCI, Bethesda, MD.

Angsubhakorn, S., Bhamarapravati, N., Romruen, K., and Sahaphong, S. (1981) Enhancing effects of dimethylnitrosamine on aflatoxin B_1 hepatocarcinogenesis in rats. Int. J. Cancer 28:621.

Asao, T., Buchi, G., Abdel-Kader, M.M. et al (1965) The structures of aflatoxins B and G_1. J. Am. Chem. Soc. 87:882.

Asplin, F.D., and Carnaghan, R.B.A. (1961) The toxicity of certain ground nut meals for poultry with special reference to their effect on ducklings and chickens. Vet. Rec. 73:1215.

Bailey, D.E., Cox, G.E., Morgareidge, K., and Taylor, J. (1976) Acute and subacute toxicity of zearalenone in the rat. Tox. Appl. Pharmacol. 37:144.

Bamburg, J.R. (1976) Chemical and biochemical studies of the trichothecene mycotoxins. In: Mycotoxins and Other Fungal Related Food Problems. J.V. Rodricks, (Ed.), Am. Chem. Soc., Washington, DC, chapter 8.

Bamburg, J.R., and Strong, F.M. (1971) 12,13-Epoxytrichothecenes. In: Microbial toxins, vol. 7. Kadis, S., Ciegler, A. and Ajl, (Eds.), Academic Press, NY, chapter 7.

Barnes, J.M., and Butler, W.H. (1964) Carcinogenic activity of aflatoxin to rats. Nature 202:1016.

Bonne, C. (1935) Cancer in Java and Sumatra. Am. J. Cancer 25:811.

Buchi, G., Foulkes, D.M., Kurono, M. and Mitchell, G.F. (1966) The total synthesis of racemic aflatoxin B_1. J. Am. Chem. Soc. 88:19.

Buchi, G. and Rae, D. (1969) The structure and chemistry of aflatoxins. In: Aflatoxin: Scientific background, control, and implications. L.A. Goldblatt, Ed., Academic Press, New York, p. 55.

Buchi, G., and Weinreb, S.M. (1969) The total synthesis of racemic aflatoxin-M_1 (milk toxin). J. Am. Chem. Soc. 91:5408.

Butler, W.H. (1964) Acute toxicity of aflatoxin B_1 in rats. Brit. J. Cancer 18:756.

Bull, L.B., Culvenor, C.C.J., and Dick, A.T. (1968) The pyrrolizidine alkaloids. In: A. Neuberger, and E.L. Tatum, Eds. Frontiers of Biology, vol. 9. Elsevier, North-Holland, Amsterdam, p. 174.

Burnside, J.E., Sippel, W.L., Forgas, J., Atwood, M.B. and Doll, E.R. (1957) A disease of swine and cattle caused by eating mouldy corn. II. Experimental production with pure cultures of mould. Am. J. Vet. Res. 18:817.

Busby, W.F. Jr., and Wogan, G.N. (1978) Food-borne mycotoxins and alimentary mycotoxicoses. In: Food-borne Infections and Intoxications, 2nd ed., H. Riemann and F. Bryan, (Eds.), Academic Press, New York, chapter 10.

Busby, W.F., and Wogan, G.N. (1981) Trichothecenes. In: Mycotoxins and N-Nitroso Compounds: Environmental Risks, Vol. II. CRC Press, Boca Raton, FL, chapter 3.

Butler, W.H. (1964) Acute toxicity of aflatoxin B_1 in rats. Brit. J. Cancer 18:756.

Butler, W.H. (1964) Acute liver injury in ducklings as a result of aflatoxin poisoning. J. Pathol. Bacteriol. 88:189.

Butler, W.H. (1966) Acute toxicity of aflatoxin B in guinea pigs. J. Pathol. Bacteriol. 91:277.

Butler, W.H. (1969) Aflatoxicosis in laboratory animals. In: Aflatoxin: Scientific background, control and implications. L. Goldblatt, Ed., Academic Press, New York, p. 223.

Butler, W.H. and Barnes, J.M. (1963) Toxic effects of groundnut meal containing aflatoxin to rats and guinea pigs. Brit. J. Cancer 17:699.

Butler, W.H., Greenblatt, M., and Lijinsky, W. (1969) Carcinogenesis in rats by aflatoxins B_1 G_1 and G_2. Cancer Res. 29:2206

Butler, W.H. and Wigglesworth, J.S. (1966) The effects of aflatoxin B_1 on the pregnant rat. Brit. J. Exptl. Pathol. 47:242.

Camargo, J.V., Punyarit, P., Rogers, A.E., and Newberne, P.M. (1983) unpublished.

Carlton, W.W., Lord, J.E. and Friedman, L. (1966) Pathology of dimethylnitrosamine poisoning in Pekin ducklings. Toxicol. Appl. Pharmacol. 8:224.

Carnaghan, R.B.A. (1965) Hepatic tumors in ducks fed a low level of toxic groundnut meal. Nature 208:308.

Carnaghan, R.B.A. Hepatic tumors and other chronic liver changes in rats following a single oral administration of aflatoxin. Brit. J. Cancer 21:811.

Carnaghan, R.B.A., Lewis, G., Patterson, D.S.P., and Allcroft, R. (1966) Biochemical and pathological aspects of groundnut

poisoning in chickens. Pathol. Vet. 3:601.

Chesterman, F.C., and Pomerance, A. (1965) Cirrhosis and liver tumours in a closed colony of golden hamsters. Brit. J. Cancer 19:802.

Chi, M.S., Mirocha, C.J., Kurtz, M.Y. et al. (1977) Acute toxicity of T-2 toxin in broiler chicks and laying hens. Poult. Sci. 56:103-116.

Christenson, C.M., Nelson, G.H., and Mirocha, A. (1965). Effect on the white rat uterus of a toxic substance isolated from Fusarium. Appl. Microbiol. 13:653.

Claridge, C.A., Bradner, W.T., and Schmitz, H. (1978) Antitumor activity of 15-acetoxyscirpen-3,4-diol. J. Antibiot. 31:485.

Claridge, C.A., and Schmitz, H. (1978) Microbial and chemical transformations of some 12,13-epoxytrichothecenes. Appl. Environ. Microbiol. 36:63.

Clegg, F.G., and Bryson, H. (1962) An outbreak of poisoning in store cattle attributed to Brazilian groundnut meal. Vet. Rec. 74:992.

Clifford, J.I., and Rees, K.R. (1967) The action of aflatoxin B_1 on rat liver. Biochem. J. 102:65.

Cundliffe, E., Cannon, M. and Davies, J. (1974) Mechanism of inhibition of eukaryotic protein synthesis by trichothecene fungal toxins. Proc. Natl. Acad. Sci. 71:30

Cundliffe, E., and Davies, J.E. (1977) Inhibition of initiation, elongation and termination of eukaryotic protein synthesis by trichothecene fungal toxins. Antimicrob. Agents Chemother. 11:491.

Cuthbertson, W.F.J., Laursen, A.C., and Pratt, D.A.H. (1967) Effect of groundnut meal containing aflatoxin on cynologus monkeys. Brit. J. Nutr. 21:893.

Detroy, R.W., Lillehoj, E.B., and Ciegler, A. (1971) Aflatoxin and related compounds. In: Microbial Toxins, Vol. VI. A. Ciegler, S. Kadis, and S.J. Ajl, Eds., Academic Press, New York, p. 4.

Donkersloot, J.A., Hsieh, D.P., and Mateles, R.I. (1968) Incorporation of precursors into aflatoxin B_1. J. Am. Chem. Soc. 90:5020.

Doyle, T.W., and Bradner, W.T. (1980) In: Anticancer Agents Based on Natural Product Models. J. Cassidy and J. Douros, eds. Academic Press, New York, chapter 2.

Dutton, M.F., and Heathcote, J.G. (1968) The structure, biochemical properties and origin of the aflatoxins B_2 and G_2. Chem. Ind. (London), p. 418.

Edwards, G., and Fox, J. (1979) Significance of nitrosamines in animal diets. Science 203:6.

Elling, F., Hold, B., Jacobsen, C., and Krogh, P. (1975) Spontaneous nephropathy in poultry associated with ochratoxin A. Acta. Path. Microbiol. Scand. Section A, 83:739.

Enomoto, M. (1959) Histopathological studies on adenomatous nodules of liver induced in mice by Penicillium islandicum Sopp. Acta. Pathol. Jap. 9:189.

Eppley, R.M., Stoloff, L., Trucksess, M.W. and Chung, C.W. (1974) Survey of corn for Fusarium toxins. J. Assoc. Off. Anal. Chem. 57:632.

Forgacs, J., and Carll, W.T. (1955) Preliminary mycotoxic studies on hemorrhagic disease in poultry. Vet. Med. 50:172.

Friedman, L., and Mobr, U. (1968) Absence of interaction between cyclopropenoid fatty acids and aflatoxin in rats. Fed. Proc. 27:551.

Garner, R.C. (1975) Reduction in binding of ^{14}C-aflatoxin B_1 to rat liver macromolecules by phenobarbitone pretreatment. Biochem. Pharmacol. 24:1553.

Goodall, C.M., and Butler, W.H. (1969) Aflatoxin carcinogenesis: Inhibition of liver cancer induction in hypophysectomized rats. Internat. J. Cancer 4:422.

Grant, K.E., Conner, M.W., and Newberne, P.M. (1977) Effect of dietary sodium selenite on lesions induced by repeated small doses of aflatoxin B_1. Toxicol. Appl. Pharmacol. 41:166.

3667

Halver, J.E. (1965) Aflatoxicosis and rainbow trout hepatoma. In: G.N. Wogan (ed.), Mycotoxins in Foodstuffs, pp. 209-234. Cambridge, MA: MIT Press.

Hansel, W., McEntee, K., and Olafson, P. (1951) The effects of two causative agents of experimental hyperkeratosis on vitamin A metabolism. Cornell Vet. 41:367.

Harwig, J. (1974) Ochratoxin A and related metabolites. In: Mycotoxins. I.F.H. Purchase, (Ed.). Elsevier, New York, chapter 16.

Higginson, J. (1963) The geographical pathology of primary liver cancer. Cancer Res. 23:1624.

Hill, L.R. (1963) Comment on the histological appearances in serial liver biopsies and postmortem specimens. Vet. Rec. 75:493.

Hoekstra, W.G., Hall, R.E., and Phillips, P.H. (1954) Relationship of vitamin A to the development of hyperkeratosis (x-disease) in calves. Am. J. Vet. Res. 15:41.

IARC - (International Agency for Research on Cancer) (1976) In: IARC Monographs on Evaluation of Carcinogenic Risk of Chemicals to Man, vol. 10, Lyon, France, p. 191.

Ishiko, T. (1957) Histological studies on the injuries of various organs except liver of mice and albino rats fed "yellowed

rice" artificially polluted by Penicillium islandicum Sopp. Acta. Pathol. Jap. 7:368.

Joseph-Bravo, P.I., Findley, M., and Newberne, P.M. (1976) Some interactions of light, riboflavin, and aflatoxin B_1 in vivo and in vitro. J. Toxicol. Environmental Health 1:353.

Kanisawa, M., and Suzuki, S. (1978) Induction of renal and hepatic tumors in mice by ochratoxin A, a mycotoxin. Gann 69:599.

Kobayashi, Y. (1958) Toxicological studies on yellowed rice by Penicillium islandicum Sopp II. Isolation of the two toxic substances from the noxious fungus, and their chemical and biological properties. Proc. Jap. Acad. 34:736.

Krogh, P. (1977) Ochratoxins. In: Mycotoxins in Human and Animal Health. J.V. Rodricks, C.W. Hesseltine, and M.A. Mehlman, (Eds.). Pathotox, Forest Park South, IL, p. 489.

Kuczuk, M.H., Benson, P.M., Heath, H., and Hayes, A.W. (1978) Evaluation of the mutagenic potential of mycotoxins using Salmonella typhimurium and Saccharomyces cerivisiae. Mutat. Res. 53:11.

Lancaster, M.C., Jenkins, F.P., and Philp, J. McL. (1961) Toxicity associated with certain samples of groundnuts. Nature (London) 192:1095.

LeBreton, E., Frayssinet, C. and Boy, J. (1962) Sur l'apparation d'hepatomes "Spontanes" chex le rat wistar: Role de la toxine de

l'Aspergillus flavus. Interet en Pathologie Humaine et Cancerologie Experimentale. Acad. Sci. (Paris) Compt. Rend. 255:784.

Lee, D.J., Wales, J.H., and Sinnhuber, R.O. (1969) Hepatoma and renal tubule adenoma in rats fed aflatoxin and cyclopropenoid fatty acids. J. Natl. Cancer Inst. 43:1037.

Lewis, G., Markson, L.M., and Allcroft, R. (1967) The effect of feeding toxic groundnut meal to sheep over a period of five years. Vet. Rec. 80:312.

Liao, L., Grollman, A.P., and Horwitz, S.B. (1976) Mechanism of action of the 12,13-epoxytrichothecene anguidine, an inhibitor of protein synthesis. Biochim. Biophys. Acta. 454:273.

Lindenfelser, L.A., Lillehoj, E.B., and Burmeister, H.R. (1974) Aflatoxin and trichothecene toxins: skin tumor induction and synergestic acute toxicity in white mice. J. Natl. Cancer Inst. 52:113.

Lintas, C., Clark, A., Tannenbaum, S.R., and Newberne, P.M. (1982) In vivo stability of nitrite and nitrosamine formation in the dog stomach: Effect of nitrite and amine concentration and of ascorbic acid. Carcinogenesis 3:161.

Loizelier, A.B. (1963) Intoxicaciones en pollos producidas por el turto de cacahuete. Rev. Patron. Biol. Animal 7:25.

Loosmore, R.M., and Harding, J.D.J. (1961) A toxic factor in Brazilian groundnut causing liver damage in pigs. Vet. Rec. 73:1362.

Loosmore, R.M., and Markson, L.M. (1961) Poisoning of cattle by Brazilian groundnut meal. Vet. Rec. 73:813.

Lovelace, C.E.A., and Nyathi, C.B. (1977) Estimation of the fungal toxins, zearalenone and aflatoxin contaminating opaque maize beer in Zambia. J. Sci. Food Agric. 28:288.

Madhavan, T.V., and Gopalan, C. (1968) The effect of dietary protein on carcinogenesis of aflatoxin. Arch. Pathol. 85:133.

Madhavan, T.V., and Gopalan, C. (1975) Effect of dietary protein on aflatoxin injury in weanling rats. Arch. Pathol. 80:123.

Madhavan, T.V., Pulpule, P.G. and Gopalan, C. (1965) Aflatoxin induced hepatic fibrosis in Rhesus monkeys. Arch. Pathol. 79:466.

Madhavan, T.V., and Rao, K.S. (1966) Hepatic infarction in ducklings in aflatoxin poisoning. Arch. Pathol. 81:520.

Madhavan, T.V., Suryanarayana, R.K., and Tulpule, P.G. (1965a) Effect of dietary protein level on susceptibility of monkeys to aflatoxin liver injury. Indian J. Med. Res. 53:984.

Magwood, S.E., Annau, E., and Corner, A.H. (1966) Induced tolerance in turkeys to aflatoxin poisoning. Can. J. Comp. Med Vet. Sci. 30:17.

Marasas, W.F., Bamburg, J.R., Smalley, E.B. et al. (1969) Toxic effects on trout, rats and mice of T-2 toxin produced by the fungus Fusarium tricinctum. Toxicol. Appl. Pharmacol. 15.471

Markson, L.M. (1960) The pathogenesis of the hepatic lesion in calves poisoned experimentally with Senecio jacobea Proc. Roy. Soc. Med. 53:283

Marsden, A.T.H. (1958) The geographical pathology of cancer in Malaya. Brit. J Cancer 12:161.

Marshall, E. (1982) Yellow rain: Filling in some of the gaps. Science 217:31.

Martin, P.M.D., and Keen, P. (1978) The occurrence of zearalenone in raw and fermented products from Swaziland and Lesotho. Gabrouaudia 16:15.

Mathieson, D.W. and Whalley, W.B. (1964) Chemistry of fungi XLIV. Conformation of citrinin. J. Chem. Soc. 4640.

McLaughlin, C.S., Vaughan, M., Campbell, I. et al. (1977). Inhibition of protein synthesis by trichothecenes. In: Hesseltine, and M. Mehlman, (Eds.), Pathotox, Park Forest South, IL, pp. 263-

McLean, A.E.M., and Marshall, A. (1971) Reduced carcinogenic effects of aflatoxin in rats given phenobarbital. Brit. J. Exptl. Path. 52:322.

Miller, J.K., Hacking, A., and Gross, V.J. (1973) Stillbirths, neonatal mortality and small litters in pigs associated with the ingestion of Fusarium toxin by pregnant sows. Vet. rec. 93:555.

Mirocha, C.J., and Christensen, C.M. (1974) Oestrogenic mycotoxins synthesized by Fusaria. In: Mycotoxins. I.F.H. Purchase, (Ed.). Elsevier, New York, chapter 6.

Mirocha, C.J., Pathre, S.V., and Christensen, C.M. (1977) Zearalenone. In: Mycotoxins in Human and Animal Health. J. Rodricks, C.W. Hesseltine, and M.A. Mehlman, (Eds.). Pathotox, Park Forest South, IL, p. 345.

Miyake, M. (1960) Toxic liver injuries and liver cirrhosis induced in mice and rats through long-term feeding with Penicillium islandicum Sopp growing rice. Acta. Pathol. Jap. 10:75.

Miyake, M. and Saito, M. (1965) Liver injury and liver tumors induced by toxins of Penicillium islandicum Sopp, growing on yellowed rice. In: Mycotoxins in Foodstuffs. G.N. Wogan, (Ed.). MIT Press, Cambridge, MA, p. 133.

National Research Council, National Academy of Sciences (1981) The Health Effects of Nitrate, Nitrite and N-nitroso Compounds. National Academy Press, Washington, DC.

Nesbitt, B.F., O'Kelly, J., Sargeant, K., and Sheridan, A. (1962) Toxic metabolites of Aspergillus flavus. Nature (London) 195:1062.

3667

Newberne, J.W., Baily, W.S., and Seibold, H.R. (1955) Notes on a recent outbreak and experimental reproduction of "Hepatitis X" in dogs. J. Am. Vet. Med. Assoc. 127:59.

Newberne, P.M. (1965) Carcinogenicity of aflatoxin-contaminated peanut meals. In: Mycotoxins in Foodstuffs, G.N. Wogan, ed. MIT Press, Cambridge, MA, p. 187.

Newberne, P.M. (1967) Biological activity of the aflatoxins in domestic and laboratory animals. Trout hepatoma research conference, U.S. Department Interior. Fish and Wildlife Service Report 70, pp. 130-144.

Newberne, P.M. (1974) Mycotoxins: toxicity, carcinogenicity and the influence of various nutritional conditions. Environ. Health Perspect. 9:1-32.

Newberne, P.M. (1976) Environmental modifiers of susceptibility to carcinogenesis. Cancer Detect. Prevent. 1:129.

Newberne, P.M. (1979) Nitrites promote lymphoma in rats. Science 294:1079.

Newberne, P.M. (1984) The influence of nutrition, immunologic status, and other factors on the development of cancer. In: D. Clayson, H. Krewski, and I. Munro, Eds., Cancer Risks and Extrapolations. (in press).

Newberne, P.M., Carlton, W.W., and Wogan, G.N. (1964) Hepatomas in rats and hepatorenal injury in ducklings fed peanut meal or

Aspergillus flavus extract. Path. Vet. 1:105.

Newberne, P.M., and Conner, M.W. (1974) Effect of selenium on acute response to aflatoxin B_1. In: D.D. Hemphill, Ed., A Symposium. Trace Substances in Environmental Health-VIII. U. of Missouri Press, Columbia, MO, p. 323.

Newberne, P.M., and Conner, M.W. (1980) Effects of sequential exposure to aflatoxin B_1 and diethylnitrosamine (DEN) on vascular, stomach and other tissues in rats. Cancer Res. 40:4037.

Newberne, P.M., deCamargo, J.L.V., and Clark, A.J. (1982) Choline deficiency, partial hepatectomy and liver tumors in rats and mice. Toxicologic Pathology 10:95.

Newberne, P.M., and Gross, R.L. (1977) The role of nutrition in aflatoxin injury. In: Mycotoxins in Human and Animal Health. J.V. Rodricks, C.W. Hesseltine, and M.A. Mehlman, Eds., Pathotox Publishers, Inc., Park Forest South, IL, p. 51.

Newberne, P.M., Harrington, D.D., and Wogan, G.N. (1966) Effects of cirrhosis and other liver insults on induction of liver tumors by aflatoxin in rats. Lab. Invest. 15:962.

Newberne, P.M., and McConnell, R.G. (1980) Toxicologic pathology: Issues and uncertainties. In: C.L. Galli, S.D. Murphy, and R. Paoletti, Eds., The Principles and Methods in Modern Toxicology. Elsevier, New York, p. 223.

3667

Newberne, P.M., and Rogers, A.E. (1973) Nutrition, monocrotaline and aflatoxin B_1 in liver carcinogenesis. Plant Foods for Man 1:23.

Newberne, P.M., and Rogers, A.E. (1973) Rat colon carcinomas associated with aflatoxin and marginal vitamin A. J. Natl. Cancer Inst. 50:439.

Newberne, P.M., and Rogers, A.E. (1976) Nutritional modulation of carcinogenesis. In: Fundamentals in Cancer Prevention, P.N. Magee et al, Eds., Univ. Tokyo Press, Tokyo, p. 15.

Newberne, P.M., and Rogers, A.E. (1981) Animal toxicity of major environmental toxins. In: Mycotoxins and N-nitroso Compounds: Environmental Risks. CRC Press, Boca Raton, FLA, pp. 51-106.

Newberne, P.M., Russo, R., and Wogan, G.N. (1966) Acute toxicity of aflatoxin B_1 in the dog. Pathol. Vet. 3:331.

Newberne, P.M., and Suphakarn, V. (1977) Preventive role of vitamin A in colon carcinogenesis in rats. Cancer 40:2553.

Newberne, P.M., and Williams, G. (1969) Inhibition of aflatoxin carcinogenesis by diethylstilbestrol in male rats. Arch. Environ. Health 19:489.

Newberne, P.M., and Wogan, G.N. (1968a) Sequential morphologic changes in aflatoxin B_1 carcinogenesis in the rat. Cancer Res. 28:770.

Newberne, P.M., and Wogan, G.N. (1968b) Potentiating effects of low protein diets on effect of effect of aflatoxin in rats. Toxicol. Appl. Pharmacol. 12:309.

Newberne, P.M., Wogan, G.N., Carlton, W.W., and Abdel-Kader, M.M. (1964) Histopathologic lesions in ducklings caused by Aspergillus flavus cultures, culture extracts and crystalline aflatoxins. Toxicol. Appl. Pharmacol. 6:542.

Newberne, P.M., Chan, W.C., and Rogers, A.E. (1974) Influence of light, riboflavin and carotene on the response of rats to the acute toxicity and monocrotaline. Toxicol. Appl. Pharmacol. 28:200.

Newberne, P.M., Hunt, C.E., and Wogan, G.N. (1967) Neoplasms in the rat associated with administration of urethan and aflatoxin. J. Exptl. Molec. Pathol. 6:285.

Newberne, P.M., Rogers, A.E., and Nauss, K.M. (1983) Choline, methionine and related factors in oncogenesis. In: Nutritional Factors in the Induction and Maintenance of Malignancy. C.E. Butterworth and M. Hutchinson, eds., Academic Press, NY, pp. 247-271.

Newberne, P.M., Rogers, A.E., and Wogan, G.N. (1968c) Hepatorenal lesions in rats fed a low lipotrope diet and exposed to aflatoxin. J. Nutr. 94:331.

Newberne, P.M., Weigert, J., and Kula, N. (1979) Effects of dietary fat on hepatic mixed function oxidases and hepatocellular

carcinoma induced by aflatoxin B_1 in rats. Cancer Res. 39:3986.

Nixon, J.E., Sinnhuber, R.O., Lee, D.J., Landers, M.K., and Harr, J.R. (1974) Effect of cyclopropenoid compounds on the carcinogenic activity of diethylnitrosamine and aflatoxin B_1 in rats. J. Natl. Cancer Inst. 53:453.

Oettle, A.G. (1964) Cancer in Africa, especially in regions south of the Sahara. J. Natl. Cancer Inst. 33:383.

Ohta, M., Matsumoto, H., Ishii, K., Ueno, Y. (1978) Potency of partially acylated trichothecenes. J. Biochem. 84:697.

Paget, G.E. (1954) Exudative hepatitis in guinea pigs. J. Pathol. Bacteriol. 47:393.

Paterson, J.S., Crook, J.C., Shand, A., Lewis, G., and Allcroft, R. (1962) Groundnut toxicity as the cause of exudative hepatitis (Oedema Disease) of guinea pigs. Vet. Rec. 74:639.

Pathre, S.V., and Mirocha, C.J. (1976) Zearalenone and related compounds. In: Mycotoxins and Other Fungal Related Food Problems. J.V. Rodricks, (Ed.). American Chemical Society, Washington, DC, chapter 10.

Patterson, D.S.P., Roberts, B.A., Shreene, B.J., Wrathall, A.E., and Gitter, M. (1977) Aflatoxin, ochratoxin and zearalenone in animal feedstuffs: Some clinical and experimental observations. Ann. Nutr. Aliment. 31:643.

Paymaster, J.C. (1964) Cancer and its distribution in India. Cancer 17:1026.

Platonow, N. (1964) Effect of prolonged feeding of toxic groundnut meal in mice. Vet. Res. 76:589.

Puchler, A. (1974) Basic problems in endemic nephropathy. In: Endemic Nephropathy. Proceedings of the 2nd International Symposium on Endemic Nephropathy, Sofia, 1972. Sofia Pub. House, Bulgarian Academy of Sciences, Sofia Bulgariz, pp. 15-18.

Purchase, I.F.H. (1967) Fungal metabolites as potential carcinogens with particular reference to their role in the etiology of hepatoma. S. Afr. Med. J. 41:406.

Raimo, H.F., Correa, R., and De Andrade, B.M. (1962) Acao toxica do fordo de torta de amendion. Produzido em Sao Paulo, Para Aves e outros animales. Bol. Ind. Animal (Sao Paulo) 20:361.

Reddy, J.K., and Svoboda, D. (1972) Effect of lasiocarpine on aflatoxin B_1 carcinogenicity in the rat. Arch. Pathol. 93:55.

Reddy, G.S., Tilak, T.B., and Krishnamurthi, D. (1973) Susceptibility of vitamin A-deficient rats to aflatoxin. Fd. Cosmet. Toxicol. 11:467.

Rogers, A.E. (1975) Variable effects of a lipotrope-deficient high fat diet on chemical carcinogenesis in rats. Cancer Res. 35:2469.

Rogers, A.E., and Newberne, P.M. (1967) The effects of aflatoxin B_1 and dimethylsulphoxide on thymidine-^3H uptake and mitosis in rat liver. Cancer Res. 27:855.

Rogers, A.E., and Newberne, P.M. (1971) Diet and aflatoxin B_1 toxicity in rats. Toxicol. Appl. Pharmacol. 20:113.

Rogers, A.E., and Newberne, P.M. (1971a) Lasiocarpine: Factors influencing its toxicity and effects on liver cell division. Toxicol. Appl. Pharmacol. 18:356.

Rogers, A.E., and Newberne, P.M. (1980) Lipotrope deficiency in experimental carcinogenesis. Nutrition and Cancer 2:104.

Ruddick, J.A., Scott, P.M., and Harmig, (1976) Teratological evaluation of zearalenone administered orally to the rat. Bull. Environ. Contam. Toxicol. 15:678.

Saffiotti, V., Montesano, R., Sellakumar, A.R., Cefis, F., and Kaufman, D.G. (1972) Respiratory tract carcinogenesis in hamsters. Cancer Res. 32:1073.

Saito, M. (1970) Suppression of 3'-Me-DAB carcinogens in rats by carcinogenic metabolites of Penicillium islandicum Sopp. In: Oncology: Proceedings, 10th International Cancer Congress, Houston, p. 25.

Saito, M., Enomoto, M., and Tatsuno, T. (1971) Yellowed rice toxins. Luteoskyrin and related compounds, chlorine-containing

compounds, and citrinin. In: Microbial Toxins, Vol. VI. A. Ciegler, S. Kadis and S.J. Ajl, (Eds.). Academic Press, New York, p. 229.

Salmon, W.D., and Newberne, P.M. (1963) Occurrence of hepatomas in rats fed diets containing peanut meal as a major source of protein. Cancer Res. 23:571.

Sargeant, K., Sheridan, A., O'Kelly, J., and Carnaghan, R.B.A. (1961) Toxicity associated with certain samples of groundnuts. Nature 192:1096.

Sargeant, K., Carnaghan, R.B.A., and Allcroft, R. (1963) Chemistry and origin. Chem. Ind. (London), p. 53.

Sargeant, K., Sheridan, A., O'Kelly, J., and Carnaghan, R.B.A. (1961) Toxicity associated with certain samples of groundnuts. Nature (London) 192:1096.

Sarkisov, A.C. 91954) Mycoses. Moscow State Publishing House for Agricultural Literature, 216 pp.

Schoental, R. (1961) Liver changes and primary liver tumours in rats given toxic guinea pig diet (M.R.C. Diet 18). Brit. J. Cancer 15:812.

Searle, C.E. (1976) Chemical carcinogens. Am. Chem. Soc. Monograph #173.

Shank, R.C. (1976) Mycotoxins in the environment. In: Trace Substances and Health: A Handbook, Part I. P.M. Newberne, (Ed.).

3667

Marcel Dekker, New York, p. 67.

Shank, R.C. (1981) Environmental toxicoses in humans. In: Mycotoxins and N-nitroso Compounds:Environmental Risks. Vol. 1. CRC Press, Boca Raton, FLA, pp. 107-140.

Shank, R.C., and Newberne, P.M. (1976) Dose-response study of the carcinogenicity of dietary nitrite and morpholine in rats and hamsters. Fd. Cosmet. Toxicol. 14:1.

Shanmugaratnam, K. (1956) Primary carcinomas of the liver and biliary tract. Brit. J. Cancer 10:232.

Shirasu, Y. (1966) Effects of pituitary hormones and prefeedug N-hydroxy-N-2-fluorenylacetamide on the metabolism of this carcinogen and on physiologic parameters. Cancer Res. 26:600.

Sikes, D., and Bridges, M.E. (1952) Experimental production of hyperkeratosis (x-disease) in cattle with a chlorinated naphthalene. Science 116:506.

Siller, W.G., and Ostler, D.C. (1961) The histopathology of an enterohepatic syndrome of turkey poults. Vet. Rec. 73:134.

Sinnhuber, R.O., Lee, D.J., Wales, J.H., and Ayres, J.L. (1968b) Dietary factors and hepatoma in rainbow trout (Salmo gairdneri) II. Cocarcinogenesis by cyclopropenoid fatty acids and the effect of gossypol and altered lipids on aflatoxin-induced liver cancer. J. Natl. Cancer Inst. 41:1293.

Sinnhuber, R.O., and Wales, J.H. (1974) Aflatoxin B_1 hepatocarcinogenicity in rainbow trout embryos. Fed. Proc. 33:274.

Sinnhuber, R.O., Wales, J.H., Ayres, J.L., Engebrecht, R.H., and Amend, D.L. (1968a) Dietary factors and hepatoma in rainbow trout (Salmo gairdneri) I. Aflatoxins in vegetable protein feedstuffs. J. Natl. Cancer Inst. 41:711.

Sporn, M.B., Dingman, C.W., Phelps, H.L., and Wogan, G.N. (1966) Aflatoxin B_1: Binding to DNA in vitro and alteration of RNA metabolism in vivo. Science 151:1539.

Steiner, P.E., and Higginson, J. (1961) Definition and classification of cirrhosis of the liver. Unio Intern. Contra Cancrum 17:581.

Steyn, P.S. (1977) Mycotoxins excluding aflatoxin zearalenone and the trichothecenes. In: Mycotoxins in Human and Animal Health. J.V. Rodricks, C.W. Hesseltine and M.A. Mehlman, (Eds.). Pathotox, Park Forest South, IL, p. 419.

Stob, M., Baldwin, R.S., Tuite, J. et al. (1962) Isolatoin of an anabolic, uterotrophic compound from corn infected with Gibberella zeae. Nature (London) 196:1318.

Suit, J.L., Rogers, A.E., Jelten, M.E.R., and Luria, S.E. (1977) Effects of diet on conversion of aflatoxin B_1 to bacterial mutagens by rats in vivo and by rat hepatic microsomes in vitro. Mutat. Res. 46:313.

3667

Suphakarn, V.S., Newberne, P.M., and Goldman, M. (1983) Vitamin A and aflatoxin: Effect on liver and colon cancer. Nutrition and Cancer 5:41.

Swenson, D.H., Lin, J.K., Miller, E.C., and Miller, J.A. (1977) Aflatoxin B_1-2,3-oxide as a probable intermediate in the covalent binding of aflatoxins B_1 to B_2 to rat liver DNA and ribosomal RNA in vivo. Cancer Res. 37:172.

Tannenbaum, S.R., Moran, D., Rand, W., Cuello, C., and Correa, P. (1979) Gastric cancer in Colombia IV. Nitrite and other ions in gastric content of residents from a high risk region. J. Natl. Cancer Inst. 62:9.

Temchareon, P. Personal communication, 1983.

Temchareon, P., Anukarakanonta, T., and Bhamarapravati, N. (1978) The influence of dietary protein and vitamin B_{12} on the toxicity and carcinogenicity of aflatoxins in rat liver. Cancer Res. 38:2185.

Tsunoda, H. (1970) Luteoskyrin. In: Toxic Microorganisms, Mycotoxins, Botulism. M. Herzberg, (Ed.). US Government Printing Office, Washington, DC, p. 143.

Ueno, Y. (1973) Akakobi toxins (Fusarium toxins II.) Food Hygiene Soc. (Japan) 14:501.

Ueno, Y. (1977) Trichothecenes: overview address. In: Mycotoxins in Human and Animal Health. J. Rodricks, C.W.

Hesseltine and M.A. Mehlman, eds. Pathotox, Park Forest South, Ill., p. 189.

Ueno, Y., and Fukushima, K. (1968) Inhibition of protein and DNA synthesis in Ehrlich ascites tumor by Nivalenol, a toxic principle of Fusarium nivale-growing rice. Experientia 24:1032.

Ueno, Y., Kubota, K., Ito, T., and Nakamura, Y. (1978) Mutagenicity of carcinogenic mycotoxins in Salmonella typhimurium research. Cancer Res. 38:536.

Ueno, Y., and Matsumoto, H. (1975) Inactivation of some thiolenzymes by trichothecene mycotoxins from Fusarium species. Chem. Pharmacol. Bull (Japan) 23:2439.

Ueno, Y., Sato, N., Thii, K., Sakai, K., Tsunoda, H., and Enomoto, M. (1973) Biological and chemical detection of trichothecene mycotoxins of Fusarium species. Appl. Microbiol. 25:699.

Ueno, Y., Ueno, I., Iitoi, Y., Tsunoda, H., Enomoto, M., and Ohtsubo, K. (1971) Toxicological approaches to the metabolites of Fusaria III. Japanese J. Exp. Med. 41:521.

UICC Technical Report Series (1982) Hepatocellular carcinoma. Report No. 17. K. Okuda and I. Mackay, eds. International Union Against Cancer, Geneva.

Uraguchi, K. (1971) Yellowed rice toxins: citreoviridin. In: Microbial Toxins, vol. 6. A Ciegler, S. Kadis, and S.J. Ajl,

(Eds.). Academic Press, New York, p. 367.

Wannop, C.C. (1961) The histopathologyof turkey "X" disease in Great Britain. Avian Diseases 5:371.

Ward, J.M., Sontag, J.M., Weisburger, E.K., and Brown, C.A. (1975) Effect of lifetime exposure to aflatoxin B_1 in rats. J. Natl. Cancer Inst. 55:107.

Wehner, F.C., Morasas, W.F.O., and Thiel, P.G. (1978) Lack of mutagenicity to Salmonella typhimurium of some Fusarium mycotoxins. Appl. Environ. Microbiol. 35:659.

Wei, C., and McLaughlin, C.S. (1974) Structure-function relationship in the 12,13-epoxytrichothecenes. Novel inhibitors of protein synthesis. Biochem. Biophys. Res. Comm. 57:838.

Wilson, B.J., Teer, P.A., Barney, G.H., and Blood, F.R. (1967) Relationship of aflatoxin to epizootics of toxic hepatitis among animals in Southern United States. Am. J. Vet. Res. 28:1217.

Wogan, G.N. (1966) Chemical nature and biological effects of the aflatoxins. Bacteriol. Rec. 30:460.

Wogan, G.N. (1968) Aflatoxin risks and control measures. Federation Proc. 27:932.

Wogan, G.N. (1973) Aflatoxin carcinogenesis. In: Methods in Cancer Research, vol. 7. H. Busch, Ed., Academic Press, New York, p. 309.

Wogan, G.N., Edwards, G.S., and Newberne, P.M. (1971) Acute and chronic toxicity of Rubratoxin B. Toxicol. Appl. Pharmacol. 19:712.

Wogan, G.N., and Newberne, P.M. (1967) Dose-response characteristics of aflatoxin B_1 carcinogenesis in the rat. Cancer Res. 27:2370.

Wogan, G.N., Paglialunga, S., and Newberne, P.M. (1974) Carcinogenic effects of low dietary levels of aflatoxin B_1 in rats. Fd. Cosmet. Toxicol. 12:681.

Yeh, S., and Cowdry, E.V. (1954) Incidence of malignant tumors in Chinese, especially in Formosa. Cancer 7:425.

Fig. 1. Liver of duckling killed 3 days after a single LD_{50} dose of aflatoxin B_1. Note biliary proliferation and lakes of fat. H and E, x 130.

Fig. 2. Liver of cow fed toxic peanut meal for 11 weeks. Note fibrosis of central vein and biliary proliferation. Picro-Mallory, x 160.

Fig. 3. Same liver as Fig. 2 showing irregularity of parenchymal nuclear size. H and E, x 400.

Fig. 4. Liver from pig killed 18 hours following a single dose of aflatoxin B_1 (2 mg/kg). Centrilobular hemorrhage and necrosis are characteristic of the acute response. H and E, x 160.

Fig. 5. Liver from pig fed toxic peanut meal for 26 weeks. Section illustrates a small regenerative nodule, fibrosis and pleomorphism of parenchymal cell nuclei. H and E, x 160.

Fig. 6. Liver of rat killed 2 days after a single LD_{50} dose of aflatoxin B_1. Periportal necrosis and bile duct hyperplasia are illustrated. H and E, x 160.

Fig. 7. Liver of rat killed 1 month after a single LD_{50} dose of aflatoxin B_1. Note residual biliary proliferation and irregularity of parenchymal cell nuclei. H and E, x 160.

Fig. 8. Liver of rat fed toxic peanut meal (5 ppm) for 10 weeks, illustrating biliary proliferation, irregularity of parenchymal nuclear size, and foci of small parenchymal cells (arrow). H and E, x 180.

Fig. 9. Area of trabecular hepatocellular carcinoma from a rat fed toxic peanut meal (5 ppm) for 12 weeks followed by 54 weeks of normal diet. H and E, x 130.

Fig. 10. Liver of guinea pig killed 2 days after a single LD_{50} dose of aflatoxin B_1. Centrilobular necrosis is primary change. H and E, x 135.

Fig. 11. Liver of guinea pig killed 7 days after a single dose of aflatoxin B_1. Note biliary proliferation radiating from periportal zone. H and E, x 135.

Fig. 12. Liver of guinea pig fed toxic peanut meal (2 ppm) for 2 weeks. Dilation of periportal lymphatics is usually severe in this species. H and E, x 135.

Fig. 13. Liver of guinea pig fed toxic peanut meal (1 ppm) for 7 weeks. Biliary proliferation and parenchymal cell

necrosis with pseudotubule formation are major morphologic alterations. H and E, x 135.

Fig. 14. Area of hepatocellular carcinoma from a guinea pig fed toxic peanut meal (0.2 ppm) for 127 weeks. A moderate vascular component is seen in this animal similar to that observed in rats. H and E, x 135.

Fig. 15. Liver of mouse fed aflatoxin for 64 weeks. Note area of hepatoma compressing normal liver at left of photograph. H and E, x 140.

Fig. 16. Liver of dog 5 days after a single dose of aflatoxin. Centrilobular necrosis and fatty change are characteristic for this species. H and E, x 190.

Fig. 17. Gallbladder of dog killed 2 days after a single dose of aflatoxin. There is extensive hemorrhage of the mucosa and edema of muscle layers and subserosa. H and E, x 12.

Fig. 18. Liver of cat killed 2 days after a single dose of aflatoxin B (0.75 mg/kg). There is early necrosis of periportal parenchymal cells. H and E, x 160.

Fig. 19. Same liver as Fig. 18, illustrating margination of nuclear chromatin of the parenchymal cells. H and E, x 360.

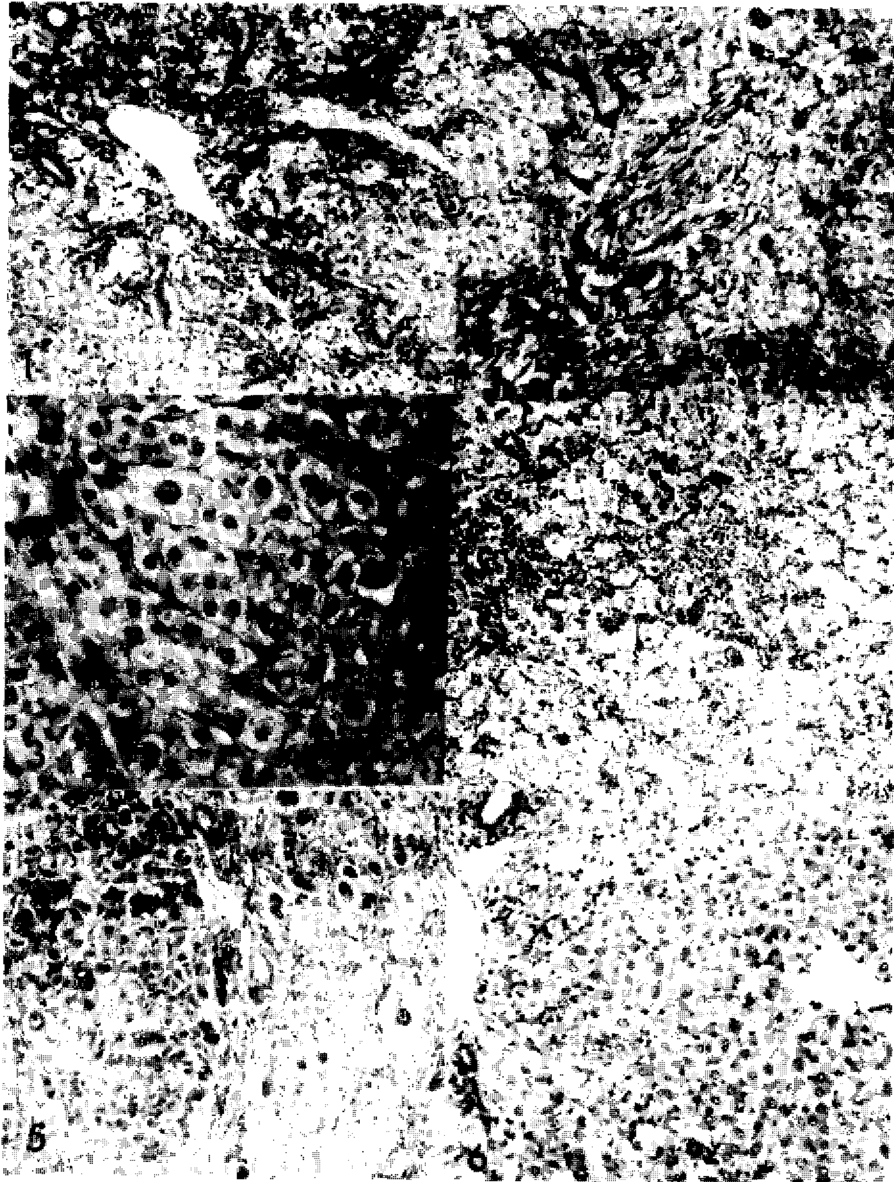
Fig. 20. Liver of rabbit killed 5 days after a single dose of aflatoxin (0.5 mg/kg). Note sharp delineation at midzonal area with hemorrhagic necrosis and with lysis of cells in the centrilobular area. H and E, x 170.

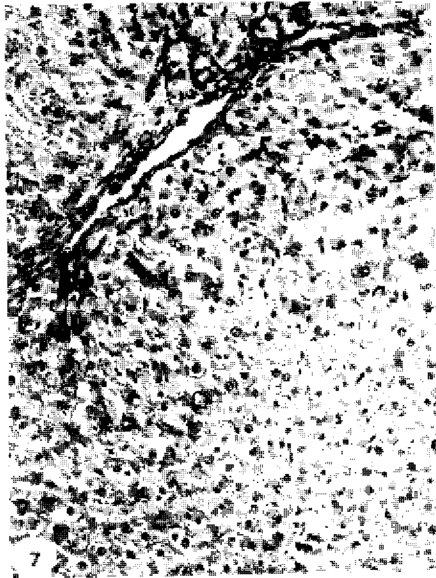
Fig. 21. Liver of monkey fed toxic peanut meal (1.8 ppm) for 3 years. There are a coarse nodular cirrhosis and large, bizarre parenchymal cells. H and E, x 135.

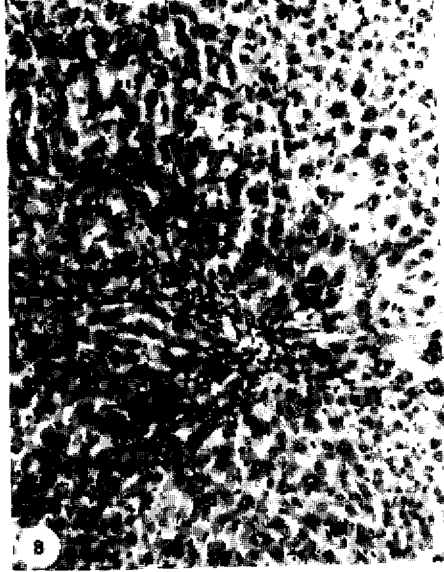
Fig. 22. Liver of trout at junction of proliferating liver cell carcinoma and more normal parenchyma. H and E, x 390.

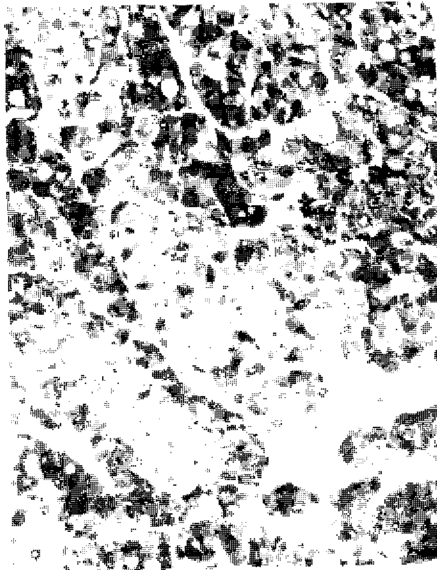
Fig. 23. Gross appearance of rat liver tumor induced by aflatoxin.

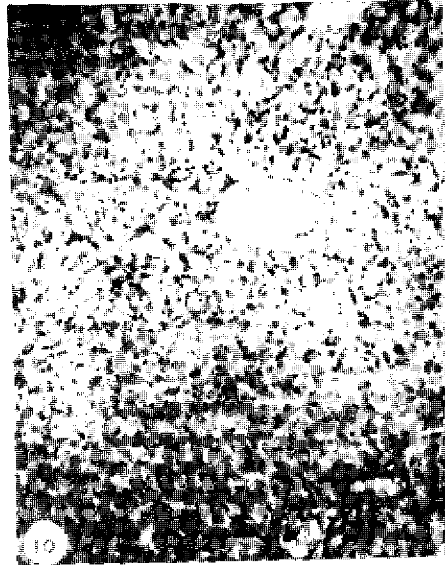
3667







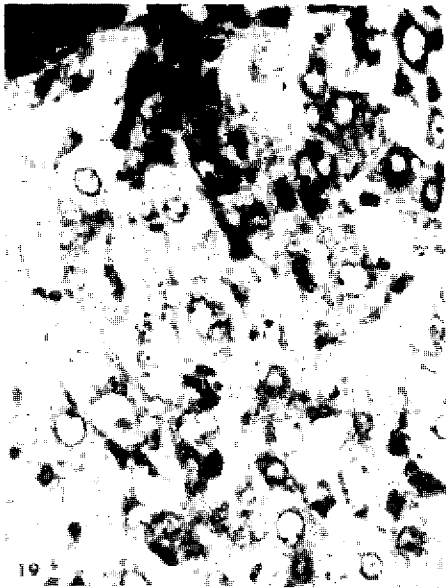


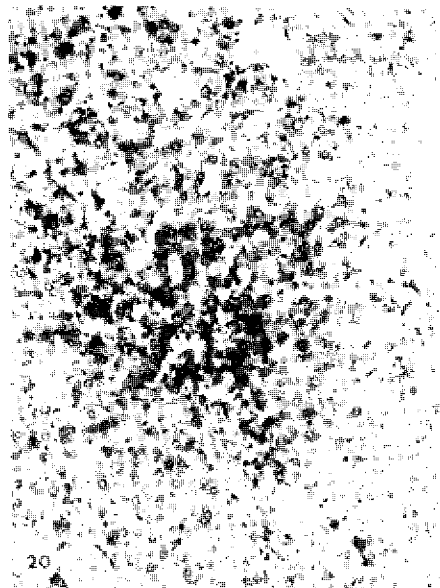


3667

I5-2

17


19


20

21

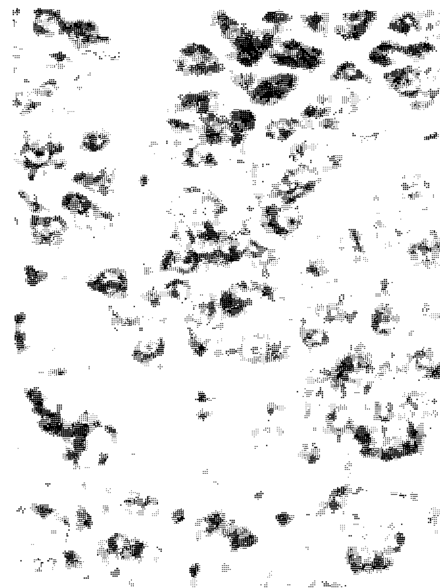

22

Table 1

Acute Toxicity of Naturally Occurring Trichothecenes[a]

Compound	LD_{50} (mg/kg body weight)	Route of Administration	Animal
T-2 toxin	3.04	intraperitoneal	mouse
T-2 toxin	3.8	peroral	rat
T-2 toxin	6.1	peroral	trout
T-2 toxin	5.25	peroral	one-day old chick[b]
nivalenol	4.0	intraperitoneal	mouse
diacetoxyscirpenol	10.0	intravenous	mouse
diacetoxyscirpenol	0.75	intraperitoneal	rat
diacetoxyscirpenol	7.3	peroral	rat

[a]From: Bamburg & Strong (1971).

[b]From: Chi et al (1977).

Table 2

Zearalenone Feed Contamination Associated With

Swine Hyperestrogenism

Sample Type	Contamination Level, mg/kg
Corn Kernels	0.1 - 0.15
Dry Sow Ration	0.15
Farrowing Ration	0.06
Lactation Ration	1.00
Gestation Ration	0.50
Mixed Feed Corn	0.12
Pelletted Feed	6.80

(From Mirocha *et al*, 1977, abridged).

Table 3

Acute Toxicity of Ochratoxin A

Species	LD_{50} mg/kg/BW	Route of Administration
Rat, male	30.3	peroral
Rat, female	21.4	peroral
Guinea pig, male	9.1	peroral
Guinea pig, female	8.1	peroral
Mouse, female	22.0	intraperitoneal
White leghorn	3.4	peroral
Turkey	5.9	peroral
Male Beagle dog	9.0	peroral
Pig, female	6.0	peroral

From Mycotoxins, UNEP, WHO, 1979, abridged.

3667

Table 4

Incidence of Liver Carcinomas in Rats Fed Aflatoxin B_1

Aflatoxin level (ppb)	Time of appearance of earliest tumor (weeks)	Duration of experiment (weeks)	Number of animals at risk[a]	Number of with hepato-cellular carcinoma
0	--	74-109	18[b]	0
1	104	78-105	22	2
5	93	65-93	22	1
15	96	69-96	21	4
50	82	71-97	25	20
100	54	54-88	28	28

[a]Animals surviving longer than 50 weeks.

[b]Animals surviving for maximum period.

(Adapted from Wogan et al., 1974).

Table 5

Dietary Protein, Vitamin B_{12} and Aflatoxin-Induced Liver Cancer in Rats

Group	Cirrhosis	Liver Tumors
Control, 5% casein	0/6	0/6
Control, 5% casein + B_{12}	0/12	0/12
5% casein + aflatoxin	21/23	3/23
5% casein + aflatoxin + B_{12}	12/24	1/24
20% casein	0/9	0/9
20% casein + B_{12}	0/10	0/10
20% casein + aflatoxin	0/24	1/24
20% casein + aflatoxin + B_{12}	0/25	6/25

(Modified from Temcharoen et al, 1978).

3667

Table 6

Vitamin A and Acute Aflatoxicosis

Sex	Vitamin A Supplement I.U./day	No. Rats	Vitamin A Content liver, I.U./ whole liver	40 hr. Mortality
Male	0	6	36 ± 6	100%
	100	6	2128 ± 153	0%
Female	0	6	19 ± 3	0%
	100	6	2300 ± 112	0%

Table 7

Vitamin A, Aflatoxin and Hepatocolon Cancer

Dietary Vitamin A mg/kg	AFB_1	No. Rats at Risk	Tumor Incidence	
			Liver	Colon
Control				
3.0	0	24	0.0	0.0
3.0	+	24	87.5	4.1
Low				
0.3	0	10	0.0	0.0
0.3	+	66	89.4	28.8
High				
30.0	0	23	0.0	0.0
30.0	+	26	92.3	7.7

From Newberne and Suphakarn, 1977, abridged.

3667

Table 8

Microsomal Protein in 12-Week-Old Rats Fed Semisynthetic Diets Since Weaning[a]

Treatment group	Microsomal Protein in liver (ug/mg)	Microsomal Protein in colon mucosa ug/mg)
Vitamin-A-deficient	14.97 ± 1.18	1.67 ± 0.29
Vitamin-A-deficient plus 125 ug AFB_1	19.42 ± 1.97[b]	3.94 ± 0.52[b]
Control	14.85 ± 0.87	2.57 ± 0.33
Control plus 125 ug AFB_1	20.91 ± 2.21[b]	3.72 ± 4.31[b]

a: Values are means ± SEM. There were seven to nine rats/group.

b: Significant difference, p = 0.001.

(From Suphakarn *et al.*, 1983, abridged).

Table 9

Microsomal BPOH and PNA Activity in Rat Colon Mucosa[a]

Treatment Group	Number of Rats	BPOH (umol/g/hr)	PNA (nmol/g/hr)
Vitamin-A-deficient	7	114 ± 15.87	63.0 ± 13.2
Vitamin-A-deficient plus 125 ug AFB_1	9	96 ± 14.00	40.8 ± 2.4[b]
Control	7	102 ± 22.67	63.0 ± 15.6
Control plus 125 ug AFB_1	7	114 ± 22.67	44.4 ± 4.2[b]

a: Values are means ± SEM.

b: Significant difference, $p = 0.05$.

(From Suphakarn *et al.*, 1983, abridged).

Table 10

Concentration of Reduced GSH in Rat Tissues[a]

Treatment group	Concentration of reduced GSH (ug/g) in liver	in colon mucosa	in small intestinal mucosa
Vitamin-A-deficient	730$\pm$60.33[b]	120$\pm$ 8.66[c]	412$\pm$40.70
Vitamin-A-deficient plus 125 ug AFB_1	521$\pm$120.90	160$\pm$14.47[d]	381$\pm$27.73
Control	1541$\pm$72.45[b]	90$\pm$5.48[c]	331$\pm$19.34
Control plus 125 ug AFB_1	1142$\pm$58.00[b]	125$\pm$9.50[c]	317$\pm$18.25

a: Values are means $\pm$ SEM. There were 11-16 rats/group.

b: $p = 0.001$.

c: $p = 0.001$.

d: $p = 0.05$.

From Suphakarn *et al*, 1983, by permission.

Table 11.

Aflatoxin-GSH Conjugate of Phenobarbital-Treated (Induced) Rats Fed Vitamin-A-Deficient or Adequate Diet[a]

Treatment Group	Aflatoxin-GSH conjugate in liver (nmol/g/hr)	Aflatoxin-GSH conjugate in colon mucosa (nmol/g/hr)
Vitamin-A-deficient	0.25(0.240-0.256)[b]	0.13(0.118-0.131)
Control	0.08(0.061-0.091)[b]	0.17(0.130-0.214)

a: Values are means, with ranges given in parentheses. There were 10 rats/group, each pretreated with phenobarbital (35 mg/kg body weight), 4 daily doses by gavage, prior to determinations. Each value in the table is from a pool sample of five rats.

b: Significant difference, $p = 0.01$.

(From Suphakarn *et al*, 1983, by permission.

Table 12

DNA Binding of AFB_1 in Colon Mucosa of Rats
Receiving Vitamin-A-Deficient or Adequate Diet[a]

Hours after AFB_1 dosing	DNA binding: Vitamin-A-deficient (nonsupplemented)	DNA binding: Vitamin-A-deficient (supplemented)	DNA binding: Control (nonsupplemented)
2	0.027–0.033		0.016–0.025
4	0.035–0.056		0.051–0.082
8	0.053–0.092		0.054–0.073
10	0.090–0.289[b]	0.251–0.373[b]	0.139–0.151
12	0.127–0.406[b]	0.051–0.172[b]	0.046–0.383
15	0.266–0.725[b]	0.064–0.121[b]	0.260–0.552
24	0.036–0.077		0.023–0.074

a: Values are ranges, expressed as picomoles DNA-bound AFB_1/A_{260} unit. There were eight rats in the 12-hour group and two rats in each of the other groups.

b: $p<0.05$.

(From Suphakarn et al, 1983, by permission).

Table 13

Selenium and Aflatoxin-Induced Liver Cancer in Rats

Dietary Selenium Content (ppm)	Nodular Hyperplasia	Hepatocellular Carcinoma
0.10	0	0/20
1.00	0	0/20
2.00	1/20	0/20
3.50	3/20	0/20
5.00	20/20	14/20

(From Newberne and McConnell, 1980).

Table 14

Protective Effects of Sodium Selenite in Aflatoxin-Treated Rats[1]

Selenium Content of Water (ppm)	Incidence of Hepatocellular Carcinoma	
	No.	%
0.05	16/19	84
0.10	11/20	55
0.50	10/19	53
1.00	4/18	22
2.00	3/19	16
3.50	10/18	55
5.00	18/19	95

1 Male Fischer rats given various levels of sodium selenite from weaning. After three weeks on study all rats dosed with 25 micrograms AFB_1 daily, 5 days per week, three weeks. All animals surviving sacrificed after 14 months.

Table 15

Dietary Fat and Aflatoxin (AFB_1) Carcinogenicity

Corn Oil		Beef Fat		Incidence of Liver Tumors After 14 Months	
During treatment with AFB_1	Following treatment with AFB_1	During treatment with AFB_1	Following treatment with AFB_1	No.	%
0	0	+	+	32	53
0	0	0	+	28	51
+	+	0	0	60	100
0	+	0	0	38	66

(From Newberne *et al*, 1979).

3667

Table 16

Effects of Diethylnitrosamine (DEN) Exposure After Administration of Aflatoxin B_1 (AFB_1)[1]

Treatment		Hepatocellular Carcinoma	Angiosarcoma Lung	Squamous[a] Carcinoma Stomach
AFB_1 (375 ug)	DEN (ppm)			
-	0	0/39	0/39	0/39
+	0	1/39	0/39	0/39
-	40	2/38	0/38	0/38
+	5	2/40	0/40	0/40
+	10	8/40	0/40	0/40
+	25	10/39	1/39	2/39
+	40	30/38	7/38	8/38

([1]From Newberne, and Conner, 1980, abridged, by permission.

Table 17

Aflatoxin and UV-irradiation (artificial sunlight) Effects on Liver Activity of Glucose-6-Phosphatase

Treatment	Glucose-6-phosphatase (umol/mg protein)
DMSO	164 ± 0.04
DMSO + AFB_1	352 ± 46.62
DMSO + AFB_1 + irradiation	309 ± 6.8

(From Joseph-Bravo *et al*, 1976, abridged, by permission).

3667

Table 18

Influence of AFB_1 and Artificial Sunlight on

Riboflavin Blood Levels

Treatment (additions)	Riboflavin Levels ug/100 ml Blood
Riboflavin	37.8 ± 6.6
Riboflavin + aflatoxin	27.6 ± 10.0
Riboflavin + aflatoxin + artificial sunlight	170.0 ± 2.6

(From Joseph-Bravo et al, 1976, abridged, by permission).

Table 19

Vehicle	Fraction	LD_{50} (mg/kg)	Reference
	B_1	0.400	Nesbitt et al. (38)
Propylene glycol	B_1	0.560	Asao et al. (4)
Dimethylformamide	B_1	0.364	Carnaghan et al.(18)
Dimethylformamide	B_2	1.696	Carnaghan et al. (18)
Dimethylformamide	G_1	0.784	Carnaghan et al. (18)
Dimethylformamide	G_2	3.440	Carnaghan et al. (18)
Dimethylformamide	B_1	0.350	Lijinsky & Butler (29)
Dimethylformamide	G_1	0.914	Lijinsky & Butler (29)

Acute LD_{50} dose of aflatoxin in ducklings.

Table 20

Animal	LD_{50} (mg/kg)	Zone of liver lesion
Duckling	0.335	Periportal
Rabbit	approx 0.3	Midzonal
Cat	0.55	Periportal
Pig	0.62	Centrilobular
Dog	approx 1.0	Centrilobular
Guinea pig	1.4	Centrilobular
Rat		
Neonate	0.56	Diffuse
Weanling	5.5	Periportal
Weanling	7.4	Periportal
100 gm	7.2	Periportal
150 gm	17.9	Periportal
Mouse	approx 9.0	
Hamster	10.2	

Summary of LD_{50} values for aflatoxin B_1.

Table 21

Sex	% toxic meal	Aflatoxin B_1 (ppm in diet)	Duration wk.(av.)	Normal diet, wk.(av.)	Liver tumors
Contaminated meal (Rossetti)[a]					
Male	50	5	36		5/6
Female	10	1	75		5/6
Female	5	0.5	88		26/33
Male	5	0.5	82		25/25
Female	1	0.1	91		5/30
Male	1	0.1	100		22/44
Male	50	5	9	54	6/6
Male	50	5	6	83	12/19
Male	50	5	3	93	3/20
Male	50	5	1	97	0/13
Purified aflatoxin B_1[b]					
Male		1	41		18/22
Female		1	64		4/4
Male		0.3	52		6/20
Female		0.3	70		11/11
Male		0.015	68		12/12
Female		0.015	82		13/13
Male		40 ug/day	10 days	82	4/24
Female		40 ug/day	10 days	82	0/23

Summary of the incidence of hepatic carcinomas in rats.

[a] W.H. Butler and J.M. Barnes, Fd. Cosmet. Toxicol., in press, 1968.

[b] G.N. Wogan and P.M. Newberne, Cancer Res., 27:2370-2376, 1967.

Table 22

Barley	Cowpeas	Sesame
Beans	Millet	Sorghum
Corn	Peas	Soybeans
Cassava	Peanuts	Sweet Potatoes
Cottonseed	Rice	Wheat

Isolated samples of food materials found to contain biologically significant amounts of aflatoxin.

Зак. 3667 ПИК ВИНИТИ