

Acute and Chronic Effects of Aflatoxin on the Liver of Domestic and Laboratory Animals: A Review^{1,2,3}

Paul M. Newberne and William H. Butler

Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, and Medical Research Council Laboratories, Toxicology Research Unit, Carshalton, Surrey, England

INTRODUCTION

The aflatoxins were isolated from peanut meal in 1961 (61) during the investigation of an epizootic of "Turkey X" disease in England. It was shown that these toxins were metabolites of some strains of *Aspergillus flavus* and that they were the etiologic agents of the disease in turkeys (7). Field outbreaks resulted from feeding peanut meal contaminated by the mold metabolites. Since these discoveries were made, the acute and chronic effects of metabolites of toxin-producing strains of *Aspergillus flavus* have been studied in detail. Investigations have shown that the metabolites consist of four major fractions, referred to collectively as "aflatoxins" and designated B₁, B₂, G₁, and G₂. Individual fractions are so designated because of their fluorescence and R_F values on thin-layer chromatographic plates. They are toxic to a large number of species, and the mixture of metabolites is carcinogenic to the rat, ferret, duck, and trout. The B₁ fraction is carcinogenic to trout and rats but less is known about the other three fractions.

This paper will describe the pathologic changes observed in the livers of different species after a single dose and after continuous administration of aflatoxin.

Turkey

Histologic changes induced in the turkey by feeds contaminated with aflatoxin have been described by Siller and Ostler (61) and by Wannop (68). 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.* (39) 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.* (58). The acute LD₅₀ of the aflatoxins has been estimated in a variety of solvents, and results from several different laboratories indicate general agreement for the B₁ fraction (see Table 1). Less is known about the other three fractions.

The lesion induced by a single dose of aflatoxin B₁ was described in detail by Butler (12). 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

¹This study was supported in part by NIH Research Grant CA 08870 and by Research Funds of the Medical Research Council, London, England.

²This manuscript is Contribution No. 1285 from the Department of Nutrition and Food Science, Massachusetts Institute of Technology.

³In addition to material supplied by the investigations of the authors, we are indebted to the following: Dr. R. Allcroft and Staff, Central Veterinary Laboratory, Weybridge, Surrey, England, for material from cattle, turkeys, and chickens, and from chronic studies with pigs; Professor J. H. Wales, Oregon State University, Corvallis, Oregon, U. S. A., for material from trout; and Dr. D. A. H. Pratt, Glaxo Research Limited, Fulmer, Buckinghamshire, England, for material from the monkey.

Received May 22, 1968; accepted September 8, 1968.

Table 1

Vehicle	Fraction	LD ₅₀ (mg/kg)	Reference
Propylene glycol	B ₁	0.400	Nesbitt <i>et al.</i> (38)
Dimethylformamide	B ₁	0.560	Asao <i>et al.</i> (4)
Dimethylformamide	B ₁	0.364	Carnaghan <i>et al.</i> (18)
Dimethylformamide	B ₂	1.696	Carnaghan <i>et al.</i> (18)
Dimethylformamide	G ₁	0.784	Carnaghan <i>et al.</i> (18)
Dimethylformamide	G ₂	3.440	Carnaghan <i>et al.</i> (18)
Dimethylformamide	B ₁	0.350	Lijinsky & Butler (29)
Dimethylformamide	G ₁	0.914	Lijinsky & Butler (29)

Acute LD₅₀ dose of aflatoxin in ducklings.

birds. At 14 days postexposure, there was an increase in mitotic activity of parenchymal cells of birds surviving an LD₅₀ dose.

Levels representing $\frac{1}{4}$ – $\frac{1}{2}$ the LD₅₀ dose resulted in less severe lesions, and there was little correlation between dose and degree of biliary proliferation. A wide variation in response to 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 LD₅₀. 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.* (16).

Asplin and Carnaghan (5) 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.* (50) 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 (37) reported hepatic infarcts in ducklings given 10–40 µg of aflatoxin a day for five days, but this has not been confirmed by other workers and appears to be uncommon. Carnaghan (17) 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 (44) 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 (5). 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 (33) and by Raimo *et al.* (56).

Carnaghan *et al.* (20) studied the experimental poisoning of chickens with toxic peanut meal contained in a diet which assayed about 1.5 ppm of the B₁ 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 (36). 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 (40). Clegg and Bryson (23) 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 pathologic alterations in the liver of cattle from India.

Allcroft and Lewis (3) 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 (28). 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.

Fig

Weanling pigs, 6–7 kg, were used to determine acute effects of aflatoxin B₁ on this species (45). Oral administration indicated an LD₅₀ 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 (35) and cases of experimental disease induced by feeding contaminated meal (24) were the same as those induced by multiple administration of mixtures of toxins (69). 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 (2) 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.* (31) 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 B₁ fraction of the aflatoxin complex. The youngest animals are most susceptible (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 (10); mature females were considerably more resistant, a characteristic which appears to be lost during the latter part of pregnancy (15).

Lesions induced by an LD₅₀ dose of aflatoxin B₁ include a periportal zone of necrosis (11) 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

Table 2

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 LD₅₀ values for aflatoxin B₁.

(1) 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 (57), 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 LD₅₀ dose of aflatoxin B₁, 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 B₁ developed hepatocellular carcinoma after two years (18).

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.* (29) showed that a diet containing meal toxic to poultry also induced hepatic carcinomas in rats. Salmon and Newberne (58) 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 (14). At high dietary levels (4–5 ppm of aflatoxin B₁) the lesion induced was 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 (14, 46), 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 (6). Subsequent investigations have attempted to establish the lowest dose level and minimal exposure time required to induce hepatic carcinomas. Using purified aflatoxin B₁, Wogan and Newberne (71) 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 B₁ 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 appar-

ent 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 B₁ administered in doses measured gravimetrically.

The sequential development of the lesions (induced by purified aflatoxin B₁) similar to those described for the contaminated meal (14) are described in detail by Newberne and Wogan (49). 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.* (54) reported the experimental induction of a disease in guinea pigs similar to a natural outbreak described by Paget more than a decade earlier (52). 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 (22) 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 B₁ in guinea pigs have been studied by Butler (13). The LD₅₀ 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 LD₅₀ 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

Table 3

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 µg/day	10 days	82	4/24
Female		40 µg/day	10 days	82	0/23

Summary of the incidence of hepatic carcinomas in rats.

^aW. H. Butler and J. M. Barnes, *Fd. Cosmet. Toxicol.*, in press, 1968.

^bG. N. Wogan and P. M. Newberne, *Cancer Res.*, 27: 2370–2376, 1967.

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 (14). 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 (55) described three-month feeding trials which failed to produce any change. Newberne (44) 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.* (48) 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 (48, 69). 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" (43), 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 seen 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 48–72 hours; grossly the liver was swollen, pale, and friable with occasional petechial hemorrhages. Microscopically, the 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 LD₅₀ 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 µg 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 (38). Cuthbertson *et al.* (24) studied the effects of contaminated peanut meal on cynomolgus 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 LD₅₀ of aflatoxin B₁ for hamsters has been reported as 10.2 mg/kg (70), but no information is available as to the pathologic changes induced. Results of preliminary feeding experiments reported by Chesterman and Pomerance (22) 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 (25) following a high incidence of hepatic tumors in hatchery-raised trout in 1960. The acute LD₅₀ of

combined aflatoxin B₁ and G₁ 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.* (43) and Burnside *et al.* (10). 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 moldy 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.* (30) 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 (43) that "hepatitis X" has now been shown to be related, at least in part, to aflatoxin-contaminated peanut meals in the diet. Paget (52) 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 (61) 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 (60), 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 (4) and racemic mixtures synthesized (9). 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 B₁ in the rat results from a capacity to bind to DNA, a characteristic similar to that of actinomycin D (23, 65). 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 LD₅₀ dose of aflatoxin B₁, 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 B₁ 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 (47), 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 (46). 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 (33, 61) 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 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 (51, 66). There are wide variations within general areas of high incidence, with the highest rate of primary hepatocellular carcinoma reported in black male Africans, particularly those from Mozambique Bantu tribes (27). 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 (53), 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 (41), Indonesia (8), South China (73), Hong Kong (where about 30% of autopsies are reported to have liver carcinoma) (W. C. Chan, personal communication), and Singapore (63) 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 etiology of primary liver cancer. We have pointed out previously (44, 46) 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 (72) 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 aflatoxin 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.

Table 4

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.

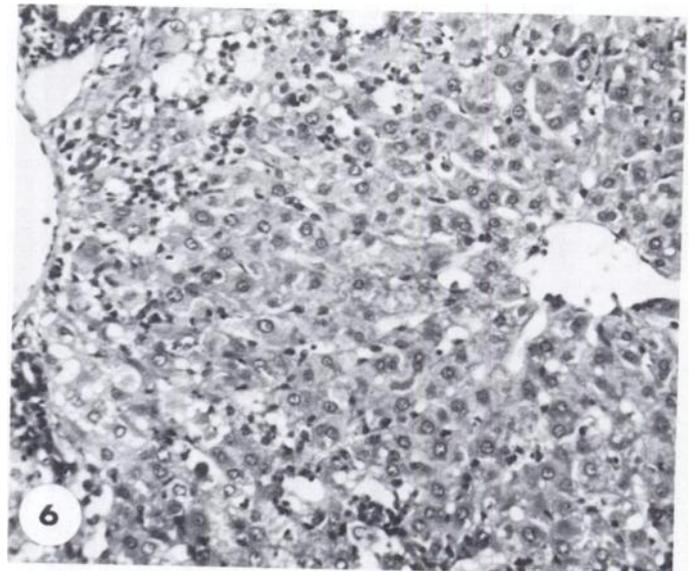
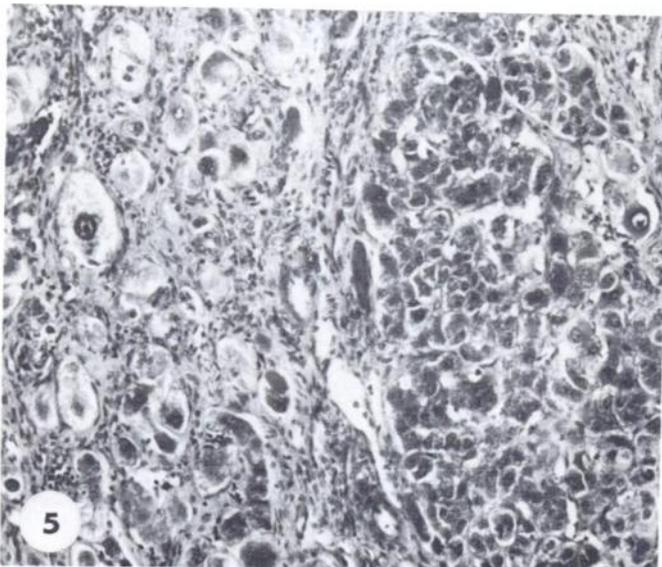
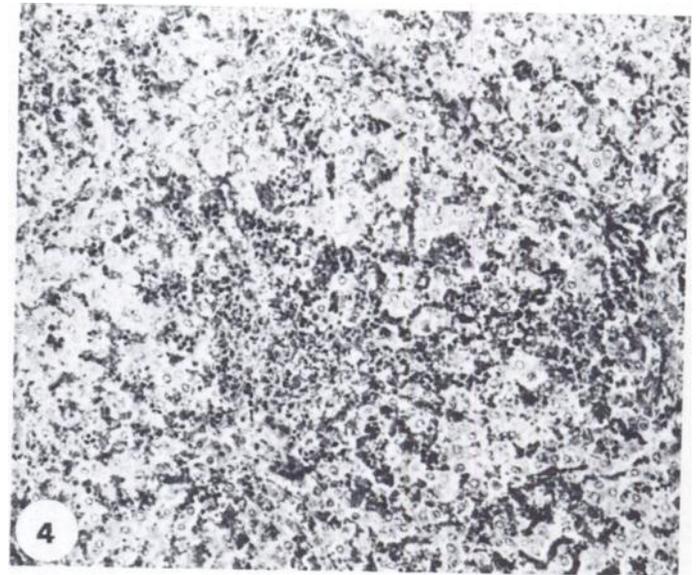
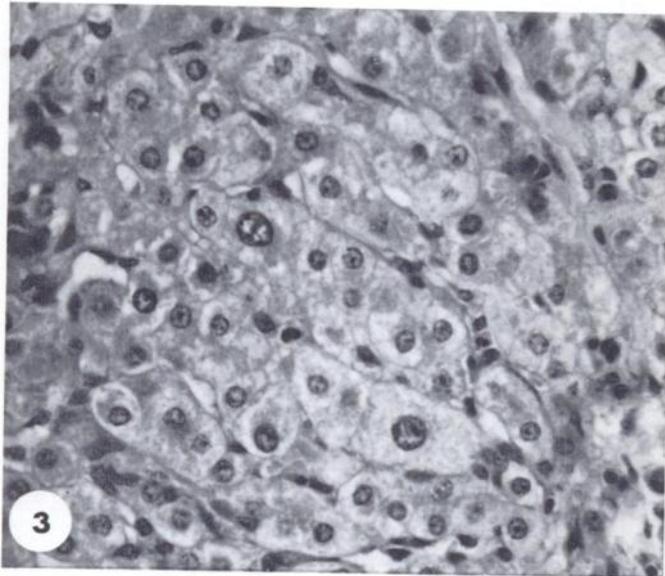
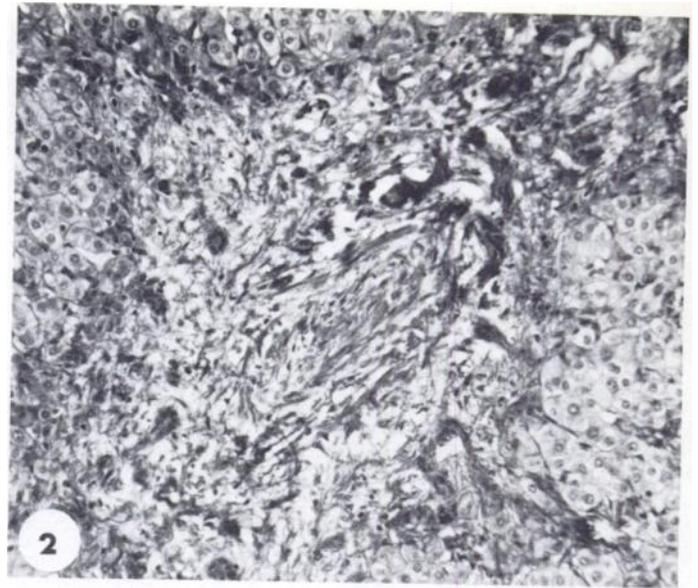
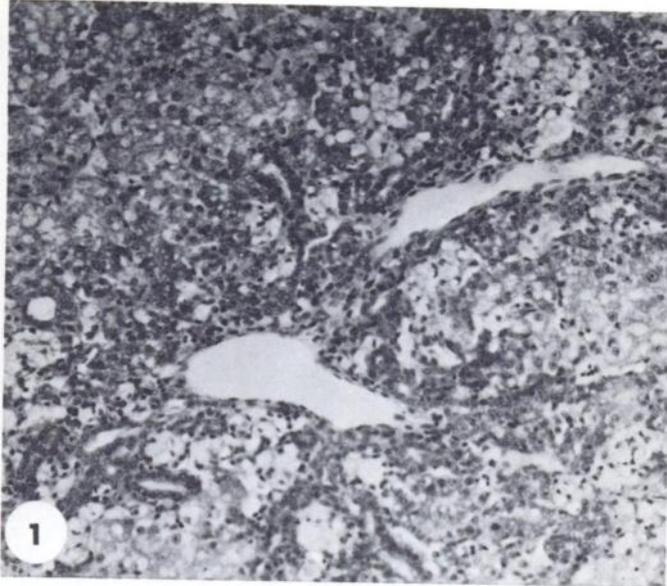
REFERENCES

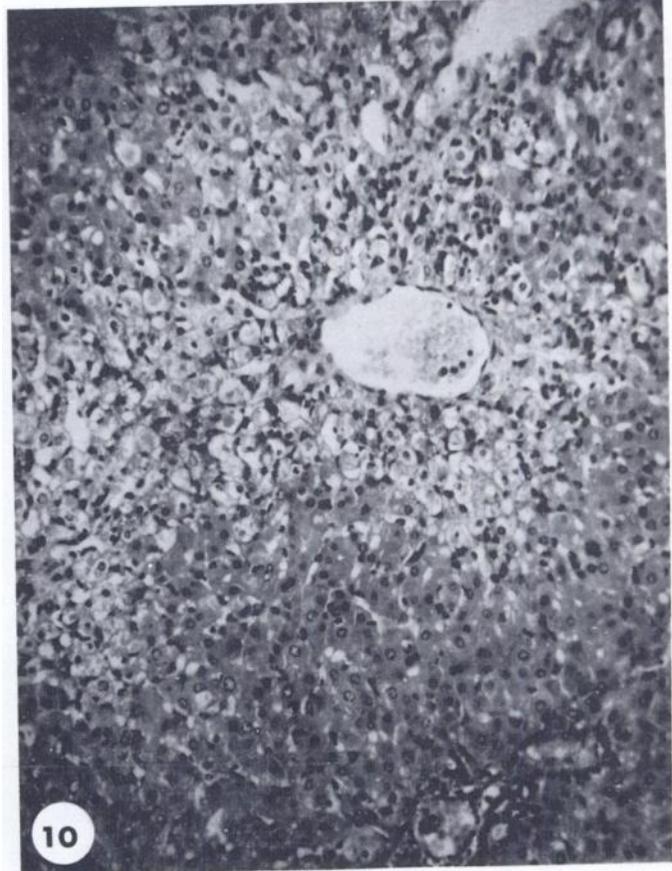
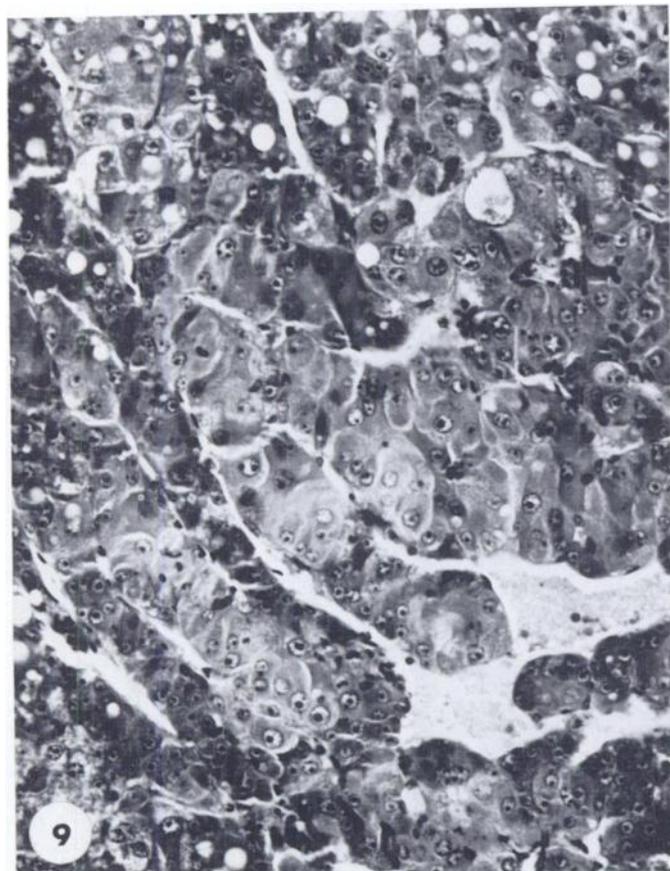
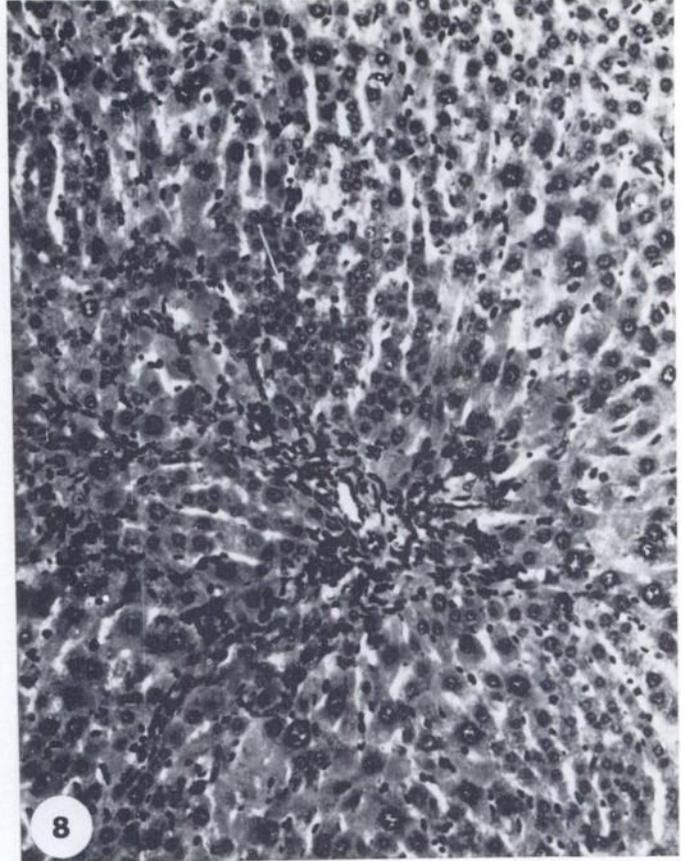
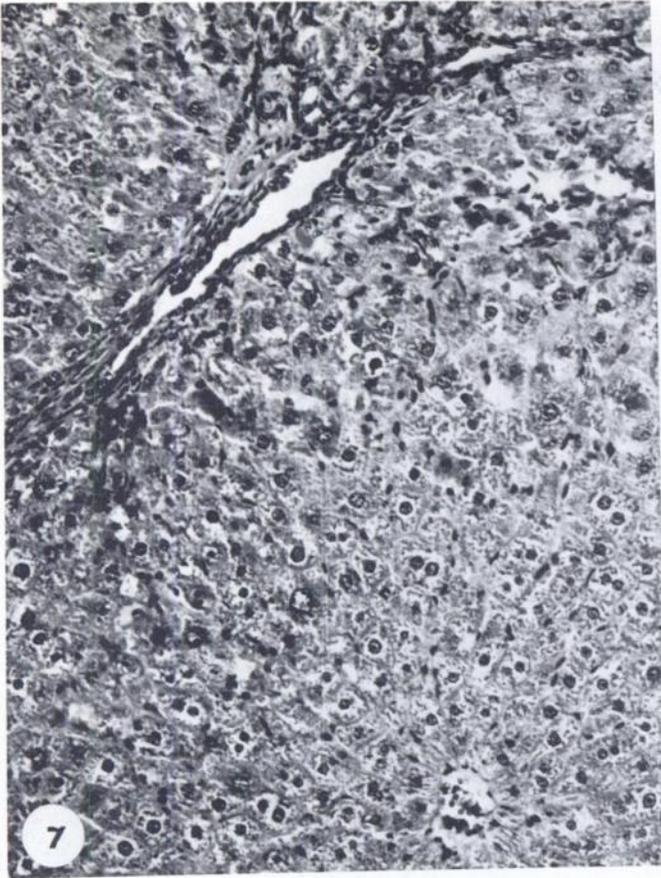
1. Abercrombie, M., and Harkness, R. D. The Growth of Cell Populations and the Properties in Tissue Culture of Segments of Liver of the Rat. *Proc. Roy. Soc. Ser. B.*, 138: 544–561, 1951.
2. Abrams, L. Mycotoxicoses. *J. S. Afr. Vet. Med. Assoc.*, 36: 5–13, 1965.
3. Allcroft, R., and Lewis, G. Groundnut Toxicity in Cattle. Experimental Poisoning of Calves and a Report on Clinical Effects in Older Cattle. *Vet. Rec.*, 75: 487–493, 1963.
4. Asao, T., Büchi, G., Abdel-Kader, M. M., Chang, S. B., Wick, E. L., and Wogan, G. N. Aflatoxins B and G. *J. Am. Chem. Soc.*, 87: 882–886, 1965.
5. Asplin, F. D., and Carnaghan, R. B. A. The Toxicity of Certain Groundnut Meals for Poultry with Special Reference to Their Effect on Ducklings and Chickens. *Vet. Rec.*, 73: 1215–1219, 1961.

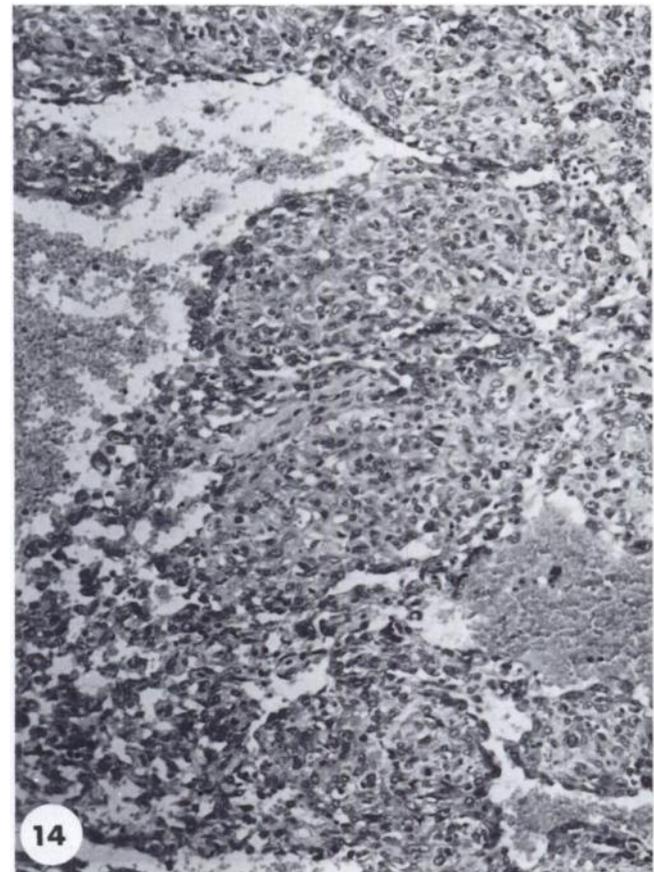
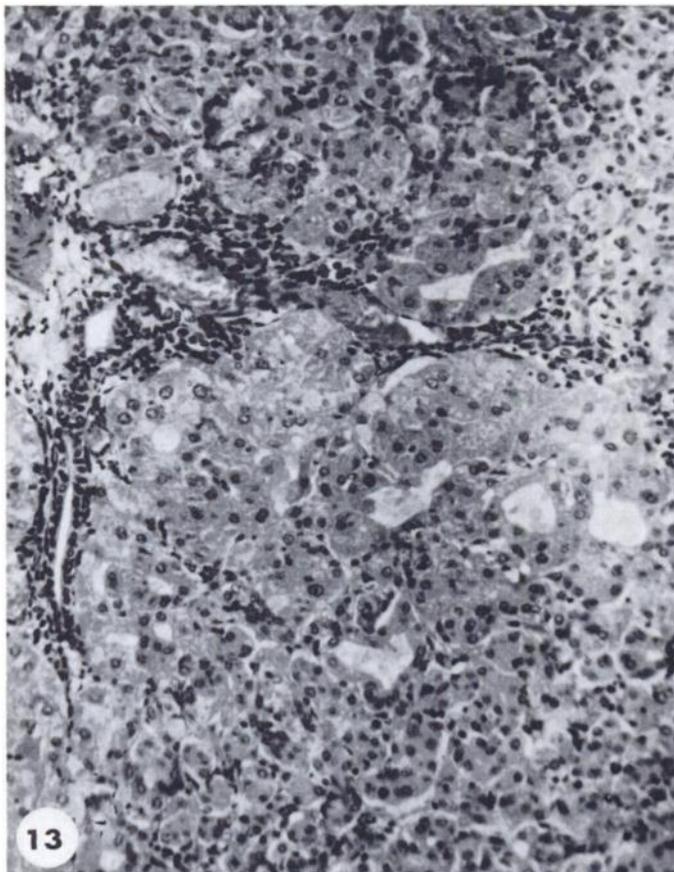
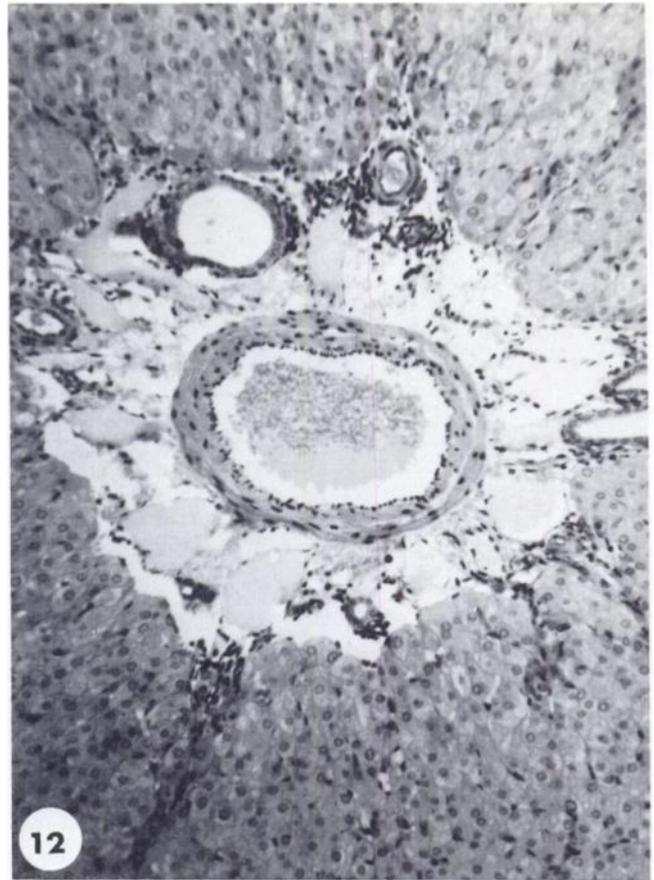
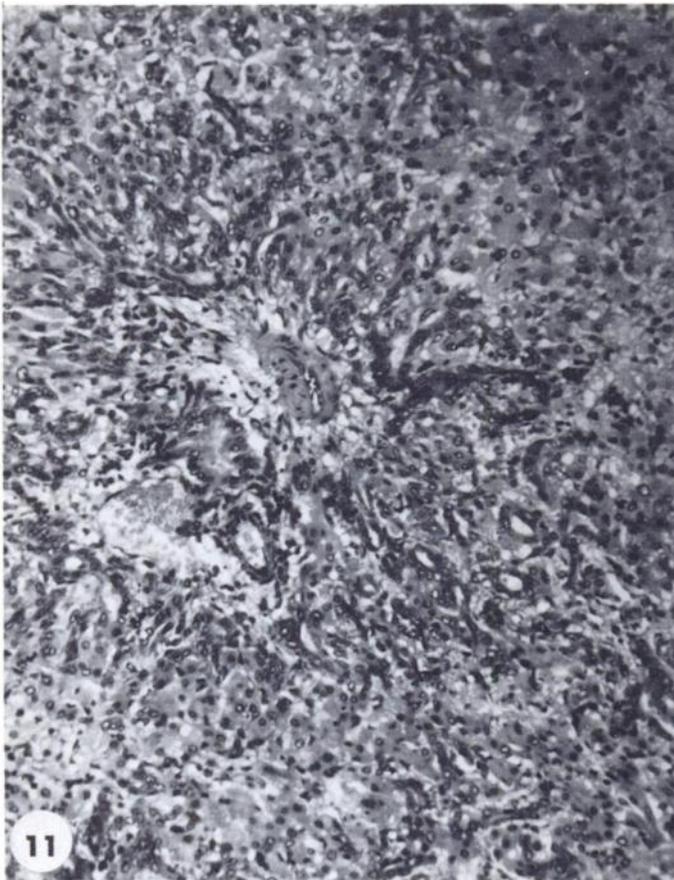
6. Barnes, J. M., and Butler, W. H. Carcinogenic Activity of Aflatoxin to Rats. *Nature*, 202: 1016, 1964.
7. Blount, W. P. Turkey "X" Disease. *Turkeys*, 9: 52, 1961.
8. Bonne, C. Cancer in Java and Sumatra. *Am. J. Cancer*, 25: 811-821, 1935.
9. Büchi, G., Foulkes, D. M., Kurono, M. and Mitchell, G. F. The Total Synthesis of Racemic Aflatoxin B₁. *J. Am. Chem. Soc.*, 88: 19, 1966.
10. Burnside, J. E., Sippel, W. L., Forgas, J., Atwood, M. B. and Doll, E. R. A Disease of Swine and Cattle Caused by Eating Mouldy Corn. II. Experimental Production with Pure Cultures of Mould. *Am. J. Vet. Res.*, 18: 817-824, 1957.
11. Butler, W. H. Acute Toxicity of Aflatoxin B₁ in Rats. *Brit. J. Cancer*, 18: 756-762, 1964.
12. Butler, W. H. Acute Liver Injury in Ducklings as a Result of Aflatoxin Poisoning. *J. Pathol. Bacteriol.*, 88: 189-196, 1964.
13. Butler, W. H. Acute Toxicity of Aflatoxin B in Guinea Pigs. *J. Pathol. Bacteriol.*, 91: 277-280, 1966.
14. Butler, W. H., and Barnes, J. M. Toxic Effects of Groundnut Meal Containing Aflatoxin to Rats and Guinea Pigs. *Brit. J. Cancer*, 17: 699-710, 1963.
15. Butler, W. H., and Wigglesworth, J. S. The Effects of Aflatoxin B₁ on the Pregnant Rat. *Brit. J. Exptl. Pathol.*, 47: 242-247, 1966.
16. Carlton, W. W., Lord, J. E., and Friedman, L. Pathology of Dimethyl-nitrosamine Poisoning in Pekin Ducklings. *Toxicol. Appl. Pharmacol.*, 8: 224-234, 1966.
17. Carnaghan, R. B. A. Hepatic Tumors in Ducks Fed a Low Level of Toxic Groundnut Meal. *Nature*, 208: 308, 1965.
18. Carnaghan, R. B. A. Hepatic Tumors and Other Chronic Liver Changes in Rats Following a Single Oral Administration of Aflatoxin. *Brit. J. Cancer*, 21: 811-814, 1967.
19. Carnaghan, R. B. A., Hartley, R. D., and O'Kelly, J. Toxicity and Fluorescence Properties of the Aflatoxins. *Nature*, 200: 1101, 1963.
20. Carnaghan, R. B. A., Lewis, G., Patterson, D. S. P., and Allcroft, R. Biochemical and Pathological Aspects of Groundnut Poisoning in Chickens. *Pathol. Vet.*, 3: 601-615, 1966.
21. Chesterman, F. C., and Pomerance, A. Cirrhosis and Liver Tumours in a Closed Colony of Golden Hamsters. *Brit. J. Cancer*, 19: 802-811, 1965.
22. Clegg, F. G., and Bryson, H. An Outbreak of Poisoning in Store Cattle Attributed to Brazilian Groundnut Meal. *Vet. Rec.*, 74: 992-994, 1962.
23. Clifford, J. I., and Rees, K. R. The Action of Aflatoxin B₁ on Rat Liver. *Biochem. J.*, 102: 65-75, 1967.
24. Cuthbertson, W. F. J., Laursen, A. C., and Pratt, D. A. H. Effect of Groundnut Meal Containing Aflatoxin on Cynologus Monkeys. *Brit. J. Nutr.*, 21: 893-908, 1967.
25. Halver, J. E. Aflatoxicosis and Rainbow Trout Hepatoma. In: G. N. Wogan (ed.), *Mycotoxins in Foodstuffs*, pp. 209-234. Cambridge, Mass.: MIT Press, 1965.
26. Harding, J. D. J., Done, J. T., Lewis, G., and Allcroft, R. Experimental Groundnut Poisoning in Pigs. *Res. Vet. Sci.*, 4: 217-229, 1963.
27. Higginson, J. The Geographical Pathology of Primary Liver Cancer. *Cancer Res.*, 23: 1624-1633, 1963.
28. Hill, L. R. Comment on the Histological Appearances in Serial Liver Biopsies and Postmortem Specimens. *Vet. Rec.*, 75: 493-494, 1963.
29. Lancaster, M. C., Jenkins, F. P., and Philp, J. McL. Toxicity Associated with Certain Samples of Groundnuts. *Nature*, 192: 1095-1097, 1961.
30. LeBreton, E., Frayssinet, C., and Boy, J. Sur l'Apparation d'Hépatomes "Spontanes" chez le Rat Wistar: Role de la Toxine de l'*Aspergillus flavus*. *Interet en Pathologie Humaine et Cancerologie Experimentale. Acad. Sci. (Paris) Compt. Rend.*, 255: 784-786, 1962.
31. Lewis, G., Markson, L. M., and Allcroft, R. The Effect of Feeding Toxic Groundnut Meal to Sheep Over a Period of Five Years. *Vet. Rec.*, 80: 312-314, 1967.
32. Lijinsky, W., and Butler, W. H. Purification and Toxicity of Aflatoxin B₁. *Proc. Soc. Exptl. Biol. Med.*, 123: 151-154, 1966.
33. Loizelier, A. B. Intoxicaciones en Pollos Producidas por el Turto de Cacahuete. *Rev. Patron. Biol.* 7: 25-33, 1963.
34. Loosmore, R. M., Allcroft, R., Tutton, E. A. and Carnaghan, R. B. A. The Presence of Aflatoxin in a Sample of Cottonseed Cake. *Vet. Rec.*, 76: 64-65, 1964.
35. Loosmore, R. M., and Harding, J. D. J. A Toxic Factor in Brazilian Groundnut Causing Liver Damage in Pigs. *Vet. Rec.*, 73: 1362-1364, 1961.
36. Loosmore, R. M., and Markson, L. M. Poisoning of Cattle by Brazilian Groundnut Meal. *Vet. Rec.*, 73: 813-814, 1961.
37. Madhavan, T. V., and Rao, K. S. Hepatic Infarction in Ducklings in Aflatoxin Poisoning. *Arch. Pathol.*, 81: 520-524, 1966.
38. Madhavan, T. V., Pulpule, P. G., and Gopalan, C. Aflatoxin Induced Hepatic Fibrosis in Rhesus Monkeys. *Arch. Pathol.*, 79: 466-469, 1965.
39. Magwood, S. E., Annau, E., and Corner, A. H. Induced Tolerance in Turkeys to Aflatoxin Poisoning. *Can. J. Comp. Med. Vet. Sci.*, 30: 17-25, 1966.
40. Markson, L. M. The Pathogenesis of the Hepatic Lesion in Calves Poisoned Experimentally with *Senecio jacobea*. *Proc. Soc. Med.*, 53: 283-284, 1960.
41. Marsden, A. T. H. The Geographical Pathology of Cancer in Malaya. *Brit. J. Cancer*, 12: 161-176, 1958.
42. Nesbitt, B. F., O'Kelly, J., Sargeant, K., and Sheridan, A. Toxic Metabolites of *Aspergillus flavus*. *Nature*, 195: 1062-1063, 1962.
43. Newberne, J. W., Baily, W. S., and Seibold, H. R. Notes on a Recent Outbreak and Experimental Reproduction of "Hepatitis X" in Dogs. *J. Am. Vet. Med. Assoc.*, 127: 59-62, 1955.
44. Newberne, P. M. Carcinogenicity of Aflatoxin-Contaminated Peanut meal. In: G. N. Wogan (ed.), *Mycotoxins in Foodstuffs*, pp. 187-208. Cambridge, Mass.: MIT Press, 1965.
45. Newberne, P. M. 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, 1967.
46. Newberne, P. M., Carlton, W. W., and Wogan, G. N. Hepatomas in Rats and Hepatorenal Injury in Ducklings Fed Peanut Meal or *Aspergillus flavus* Extract. *Pathol. Vet.* 1: 105-132, 1964.
47. Newberne, P. M., Harrington, D. D., and Wogan, G. N. Effects of Cirrhosis and Other Liver Insults on Induction of Liver Tumors by Aflatoxin in Rats. *Lab. Invest.*, 15: 962-969, 1966.
48. Newberne, P. M., Russo, R., and Wogan, G. N. Acute Toxicity of Aflatoxin B₁ in the Dog. *Pathol. Vet.*, 3: 331-340, 1966.
49. Newberne, P. M., and Wogan, G. N. Sequential Morphologic Changes in Aflatoxin B₁ Carcinogenesis in the Rat. *Cancer Res.*, 28: 770-781, 1968.
50. Newberne, P. M., Wogan, G. N., Carlton, W. W., and Abdel-Kader, M. M. Histopathologic Lesions in Ducklings Caused by *Aspergillus flavus* Cultures, Culture Extracts and Crystalline Aflatoxins. *Toxicol. Appl. Pharmacol.*, 6: 542-556, 1964.
51. Oetlé, A. G. Cancer in Africa, Especially in Regions South of the Sahara. *J. Natl. Cancer Inst.*, 33: 383-439, 1964.
52. Paget, G. E. Exudative Hepatitis in Guinea Pigs. *J. Pathol. Bacteriol.*, 47: 393, 1954.
53. Paymaster, J. C. Cancer and Its Distribution in India. *Cancer*, 17: 1026-1034, 1964.
54. Paterson, J. S., Crook, J. C., Shand, A., Lewis, G., and Allcroft, R.

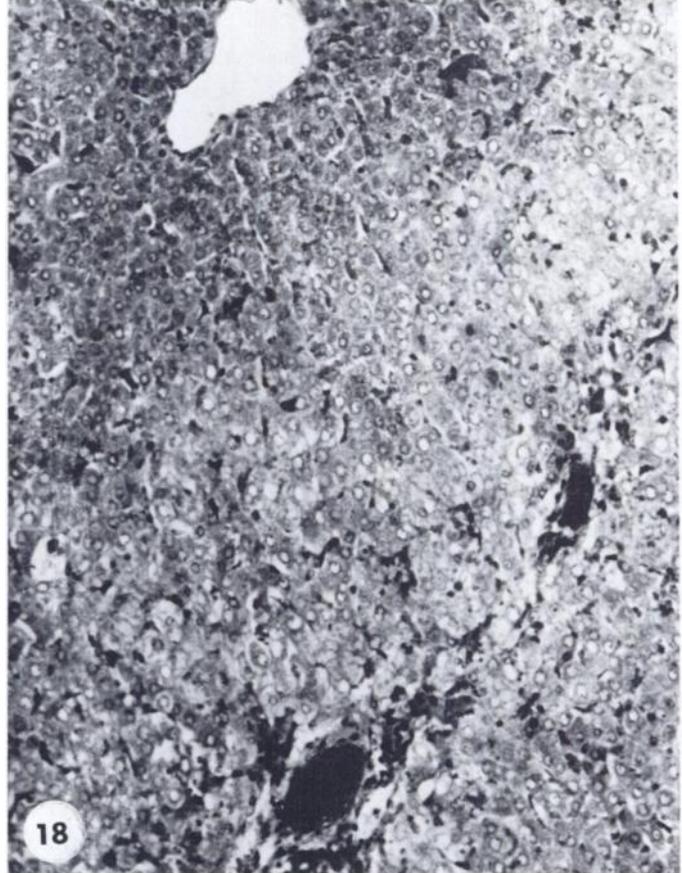
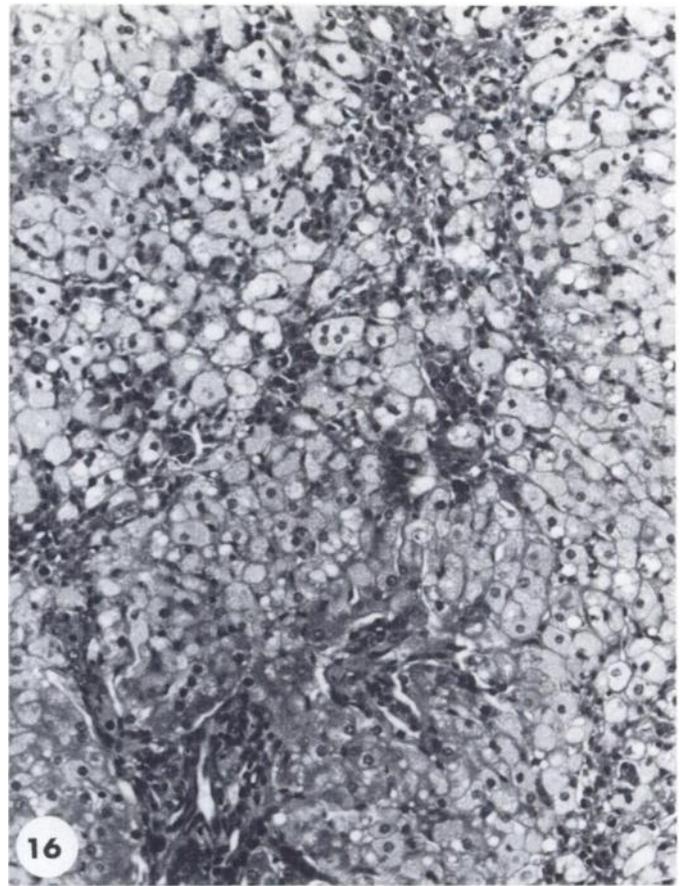
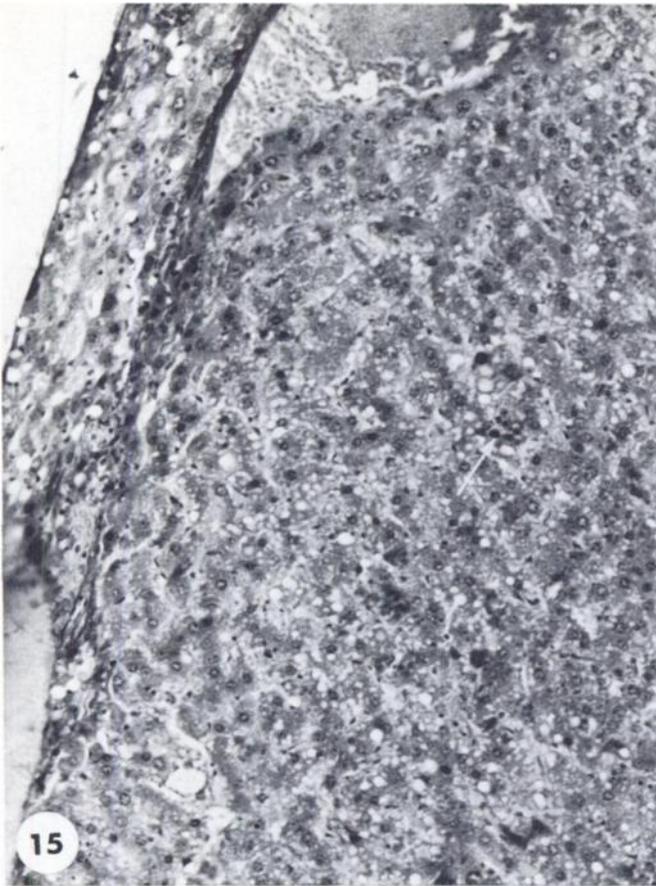
- Groundnut Toxicity as the Cause of Exudative Hepatitis (Oedema Disease) of Guinea Pigs. *Vet. Rec.*, 74: 639–640, 1962.
55. Platonow, N. Effect of Prolonged Feeding of Toxic Groundnut Meal in Mice. *Vet. Rec.*, 76: 589–590, 1964.
56. Raimo, H. F., Correa, R., and De Andrade, B. M. Acao Toxica do Fardo de Torta de Amendion. Produzido em Sao Paulo, Para Aves e outros Animales. *Bol. Ind. Animal (Sao Paulo)* 20: 361–364, 1962.
57. Rogers, A. E., and Newberne, P. M. The Effects of Aflatoxin B₁ and Dimethylsulphoxide on Thymidine-³H Uptake and Mitosis in Rat Liver. *Cancer Res.*, 27: 855–864, 1967.
58. Salmon, W. D., and Newberne, P. M. Occurrence of Hepatomas in Rats Fed Diets Containing Peanut Meal as a Major Source of Protein. *Cancer Res.*, 23: 571–575, 1963.
59. Sargeant, K., O'Kelly, J., Carnaghan, R. B. A., and Allcroft, R. The Assay of a Toxic Principle in Certain Groundnut Meals. *Vet. Rec.*, 73: 1219–1223, 1961.
60. Sargeant, K., Sheridan, A., O'Kelly, J., and Carnaghan, R. B. A. Toxicity Associated with Certain Samples of Groundnuts. *Nature*, 192: 1096–1097, 1961.
61. Schoental, R. Liver Changes and Primary Liver Tumours in Rats Given Toxic Guinea Pig Diet (M.R.C. Diet 18). *Brit. J. Cancer*, 15: 812–815, 1961.
62. Sellschop, J. P. F., Kriek, N. P. J., and du Preez, J. C. G. Distribution and Degree of Occurrences of Aflatoxin in Groundnuts and Groundnut Products. *S. Afr. Med. J.*, 39: 771–774, 1965.
63. Shanmugaratnam, K. Primary Carcinomas of the Liver and Biliary Tract. *Brit. J. Cancer*, 10: 232–246, 1956.
64. Siller, W. G., and Ostler, D. C. The Histopathology of an Enterohepatic Syndrome of Turkey Poults. *Vet. Rec.*, 73: 134–138, 1961.
65. Sporn, M. B., Dingman, C. W., Phelps, H. L., and Wogan, G. N. Aflatoxin B₁: Binding to DNA *in vitro* and Alteration of RNA Metabolism *in vivo*. *Science*, 151: 1539–1541, 1966.
66. Steiner, P. E., and Higginson, J. Definition and Classification of Cirrhosis of the Liver. *Unio Intern. Contra Cancrum*, 17: 581–592, 1961.
67. Sutton, P. M., and Spurgeon, P. J. Mitoses in Bile Duct Epithelium Following Acute Carbon Tetrachloride Poisoning. *Brit. J. Exptl. Pathol.*, 47: 545–549, 1966.
68. Wannop, C. C. The Histopathology of Turkey "X" Disease in Great Britain. *Avian Diseases*, 5: 371–381, 1961.
69. Wilson, B. J., Teer, P. A., Barney, G. H., and Blood, F. R. Relationship of Aflatoxin to Epizootics of Toxic Hepatitis Among Animals in Southern United States. *Am. J. Vet. Res.*, 28: 1217–1230, 1967.
70. Wogan, G. N. Chemical Nature and Biological Effects of the Aflatoxins. *Bacteriol. Rec.*, 30: 460–470, 1966.
71. Wogan, G. N., and Newberne, P. M. Dose-Response Characteristics of Aflatoxin B₁ Carcinogenesis in the Rat. *Cancer Res.*, 27: 2370–2376, 1967.
72. Wogan, G. N. Aflatoxin Risks and Control Measures. *Federation Proc.*, 27: 932–938, 1968.
73. Yeh, S., and Cowdry, E. V. Incidence of Malignant Tumors in Chinese, Especially in Formosa. *Cancer*, 7: 425–436, 1954.

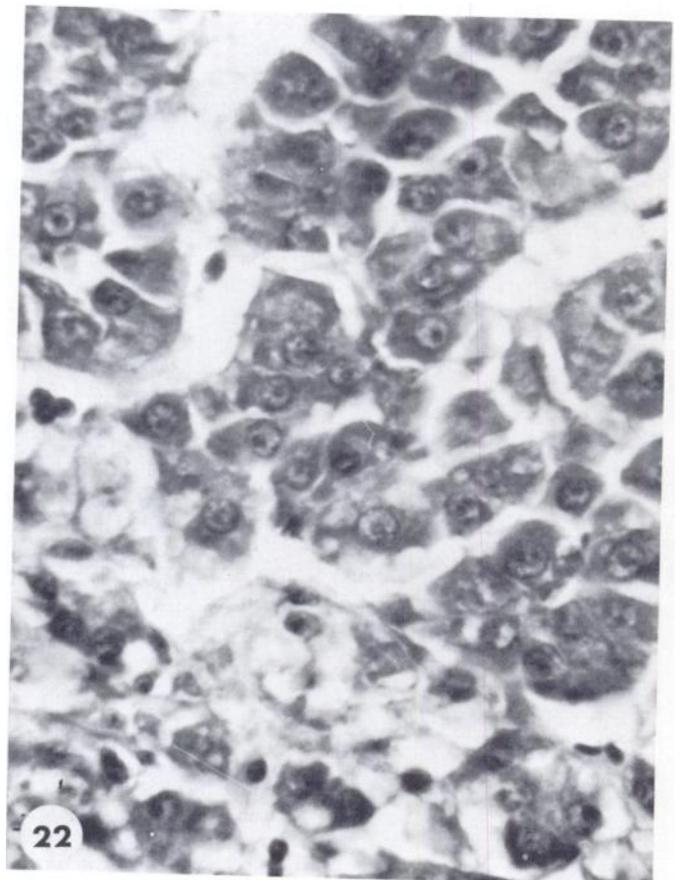
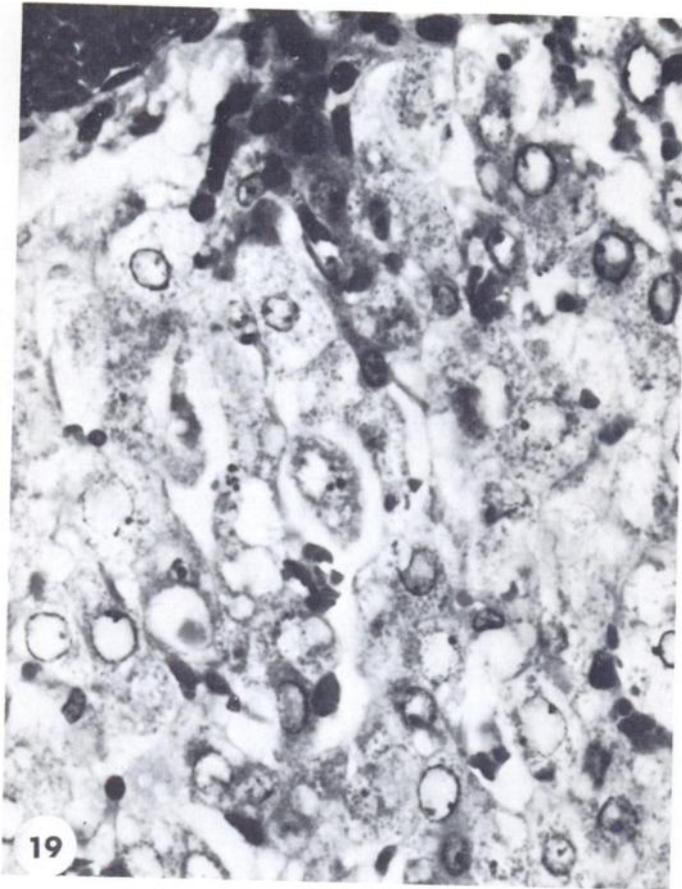
- Fig. 1. Liver of duckling killed 3 days after a single LD₅₀ dose of aflatoxin B₁. Note biliary proliferation and lakes of fat. H and E, × 130.
- Fig. 2. Liver of cow fed toxic peanut meal for 11 weeks. Note fibrosis of central vein and biliary proliferation. Picro-Mallory, × 160.
- Fig. 3. Same liver as Fig. 2 showing irregularity of parenchymal nuclear size. H and E, × 400.
- Fig. 4. Liver from pig killed 18 hours following a single dose of aflatoxin B₁ (2 mg/kg). Centrilobular hemorrhage and necrosis are characteristic of the acute response. H and E, × 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, × 160.
- Fig. 6. Liver of rat killed 2 days after a single LD₅₀ dose of aflatoxin B₁. Periportal necrosis and bile duct hyperplasia are illustrated. H and E, × 160.
- Fig. 7. Liver of rat killed 1 month after a single LD₅₀ dose of aflatoxin B₁. Note residual biliary proliferation and irregularity of parenchymal cell nuclei. H and E, × 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, × 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, × 130.
- Fig. 10. Liver of guinea pig killed 2 days after a single LD₅₀ dose of aflatoxin B₁. Centrilobular necrosis is primary change. H and E, × 135.
- Fig. 11. Liver of guinea pig killed 7 days after a single dose of aflatoxin B₁. Note biliary proliferation radiating from periportal zone. H and E, × 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, × 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, × 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, × 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, × 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, × 190.
- Fig. 17. Gall bladder 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, × 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, × 160.
- Fig. 19. Same liver as Fig. 18, illustrating margination of nuclear chromatin of the parenchymal cells. H and E, × 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, × 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, × 135.
- Fig. 22. Liver of trout at junction of proliferating liver cell carcinoma and more normal parenchyma. H and E, × 390.











Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

Acute and Chronic Effects of Aflatoxin on the Liver of Domestic and Laboratory Animals: A Review

Paul M. Newberne and William H. Butler

Cancer Res 1969;29:236-250.

Updated version Access the most recent version of this article at:
<http://cancerres.aacrjournals.org/content/29/1/236.citation>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.