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Fd Cosmet. Toxicol. Vol 16, pp 449 461
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0015-6264/78/1001-0449$02.00/0

MYCOTOXICOSIS PRODUCED IN RATS BY CULTURAL PRODUCTS OF AN ISOLATE OF *ASPERGILLUS OCHRACEUS*

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(*Received 9 May 1978*)

Abstract—The toxicity of an isolate of *Aspergillus ochraceus* was examined in weanling male Sprague–Dawley rats fed diets containing a rice culture or fungal mat of the organism for 5 wk. The ground rice culture was mixed with a commercial purified diet at concentrations of 5, 7·5 and 10% and the fungal mat at concentrations of 1, 2 and 3%. In groups fed the rice-culture diets, weight gains were reduced and all the rats in the group fed the 10% diet died. Rats fed fungal-mat diets showed a marked reduction in weight gain, and the death rate was high (about 83%) in groups fed the 2 or 3% diet. Gross lesions found in groups fed either type of diet included focal necrosis in the liver, greenish discoloration of the kidneys, gastric ulceration, ulceration of the scrotal epidermis and corneal opacity. Histological changes in the liver included necrosis of the epithelium of biliary ducts, periductal oedema, pericholangitis, periductal fibrosis and disseminated focal hepatocellular necrosis. Necrosis of epithelium occurred also in the extraheptic ducts and was accompanied by an interstitial pancreatitis in certain rats. Foci of leucocytes and macrophages were found in the dermis of the scrotum and in the epididymal adipose connective tissue. Ulceration of the epidermis occurred late in the sequence of scrotal changes. Ocular lesions comprised corneal oedema, interstitital keratitis, iridocyclitis and hypopyon. Renal lesions consisted of tubular necrosis and hyaline and biliary casts within convoluted tubules.

INTRODUCTION

Aspergillus ochraceus has frequently been isolated from various foods, including heating grains (Wallace & Sinha, 1962), Japanese polished and unpolished rice (Udagawa, Ichinoe & Kurata, 1970), pepper (Christensen, Fanse, Nelson, Bates & Mirocha, 1967; Leyendecker, 1954), pecans (Doupnik & Bell, 1971) and Mexican corn (Lopez & Christensen, 1967), but has only been isolated infrequently from US stored corn. *A. ochraceus* may elaborate several mycotoxins, including ochratoxin A and penicillic acid (Ciegler, 1972; Hesseltine, Vandegraft, Fennell, Smith & Shotwell, 1972; Munro, Scott, Moodie & Willes, 1973; Natori, Sakaki, Kurata, Udagawa, Ichinoe, Saito & Umeda, 1970; van der Merwe, Steyn, Fourie, Scott & Theron, 1965) and these two mycotoxins may be produced singly or in combination not only by *A. ochraceus* but also by other members of the *A. ochraceus* group (Ciegler, 1972).

An isolate of *A. ochraceus* (Zimmermann, Carlton & Tuite, 1976) was found to be toxic when fed to mice, the hepatic lesions produced consisting of bile-duct necrosis, periductal inflammation and fibrosis and bile-duct hypertrophy and hyperplasia. Renal lesions comprised necrosis of the convoluted tubules and the presence of biliary casts within the convoluted tubules and collecting ducts. This spectrum of lesions was similar to that observed in mice and rats fed an Indiana isolate of *Penicillium viridicatum* (Budiarso, Carlton & Tuite, 1971; Carlton, Tuite & Mislivec, 1968 & 1970). This report describes the mycotoxicosis produced in rats by cultural products of an isolate of *A. ochraceus*.

EXPERIMENTAL

Rice cultures and fungal mats of *A. ochraceus* were prepared as previously described (Zimmermann *et al.* 1976) and mixed with a purified diet. Rice culture at dietary concentrations of 5, 7·5 and 10% and ground fungal mat at concentrations of 1, 2 and 3% were fed to groups of 12 male weanling Sprague–Dawley rats for a period of 5 wk. Control rats were fed a purified diet mixed with 10% rice treated with 1% propionic acid. Rats were housed in individual stainless-steel cages in an environmentally controlled room with the temperature maintained between 21 and 24°C. Water and feed were provided *ad lib.* The rats were weighed weekly. Rats that died, those killed when moribund and those killed when the experiments were terminated after 5 wk were autopsied. The liver, kidney, stomach, duodenum, pancreas, spleen and mesenteric lymph nodes, scrotum and eye were fixed in 10% buffered formalin, processed for paraffin sectioning, and stained with haematoxylin and eosin for histopathological examination.

RESULTS

Clinical signs

Diets containing rice culture or fungal mat were toxic to rats and resulted in reduced weight gains at all dietary concentrations and deaths in all but one group (Table 1). Rats fed the highest dietary concentration of rice culture (10%) did not survive beyond 2 wk, although there were few or no deaths in the groups fed the 7·5 and 5% concentrations. A high death rate was observed in groups fed the fungal-mat diets at concentrations of 2 or 3%, but few rats died in the group fed the 1% diet. Weight gains were markedly reduced in groups fed diets containing 2 or 3% fungal mat.

Rats fed either rice-culture or fungal-mat diets developed roughened hair, became anorectic and were dehydrated, and several from each dietary group developed grossly observable scrotal necrosis. By inspection, the LD_{50} for rats fed rice culture was determined to be between 7·5 and 10% and for those fed the fungal mat the LD_{50} was between 1 and 2%.

Gross pathology

Gross lesions in rats fed either rice culture or fungal mat were generally restricted to focal lesions in the liver, greenish discoloration of the kidneys and necrosis of the scrotal skin. Occasionally, rats fed either diet developed unilateral or bilateral corneal opacity. Early changes in the liver were a variation in colour from dark red to pale tan with accentuation of the lobular patterns. Rats autopsied after 7–10 days of feeding had variably-sized green pinpoint or larger foci throughout the various lobes. The majority were just under the capsule, although a few were visible on the cut surface. Gastric ulceration was found in a few rats fed either the fungal-mat or rice-culture diets (Table 2). These gastric lesions ranged from pinpoint craters to areas several millimeters in diameter within the glandular portion of the stomach. The mucosa surrounding the lesions was often swollen and was covered with considerable mucous. The majority of scrotal lesions occurred after 2 wk and were evident initially as dark areas about the midline accompanied by partial alopecia. Subsequently, small round to elliptical lesions followed the initial oedematous phase; these were often covered by a thin reddish scab which later became thickened and brownish-black. Additional foci developed, both laterally and posteriorly, and with expansion and coalescence the entire scrotal skin became necrotic. Occasionally linear areas of necrosis progressed anteriorly from the groin towards the flank, and rarely, small necrotic foci were found on the medial aspects of the thigh and on the tailhead. The penis and penile sheath were free of lesions. Most rats with necrosis of the scrotal skin also had a variable degree of epididymal fat necrosis. Grossly, involvement of the testes was not observed.

Table 1. *Average weight gains and numbers of deaths in groups of 12 male rats fed a rice culture or fungal mat of* Aspergillus ochraceus

Test material and dietary level (%)	Mean body weight (g)		No. of deaths
	Initial	Final (wk 5)	
Rice culture			
0*	54	248	0
5	54	165	0
7.5	56	110	3
10	55	—†	12
Fungal mat			
0*	52	212	0
1	57	170	1
2	55	80	10
3	58	85	10

* Control rats were fed a purified diet mixed with 10% rice.
† No survivors at wk 5.

Gross ocular alterations occurred in three rats fed the rice-culture diets and in four fed the fungal-mat diets. These changes were characterized early by either unilateral or bilateral loss of corneal transparency and later by a slight greyish opacity of the cornea. Corneal changes usually began after 2 wk on either the rice-culture or fungal-mat diet and most regressed within 7–10 days.

Grossly, kidneys with lesions showed a greenish discoloration or were enlarged and pale.

Microscopic pathology

The severity and numbers of lesions were more variable in rats fed the fungal-mat diets than in the groups fed the rice-culture diets. This variation was due in part to the early death of the rats fed the highest concentration of rice culture. The highest incidence and greatest severity of hepatic and renal lesions occurred in rats fed the two higher fungal-mat concentrations (Tables 3 and 4).

The earliest histological alterations in the liver consisted of variable numbers of mononuclear inflammatory cells within the portal areas and a proliferation of biliary epithelium, which formed short columns and aggregates of cells (Fig. 1). These changes occurred consistently in association with a necrotizing cholangitis characterized by flattening and necrosis of the epithelium of the intrahepatic bile ducts (Fig. 2). In some livers, the bile-duct necrosis was accompanied by periductal oedema and infiltration of the oedematous tissue by inflammatory cells (Fig. 3). In severely affected livers, most of the portal areas contained necrotic bile ducts and in some portal areas the portal vein and hepatic artery and the surrounding hepatic parenchyma were necrotic as well (Fig. 4). In occasional rats fed the 3% fungal-mat diet, necrotic foci had coalesced to produce areas of necrosis that incorporated several contiguous lobules. In rats that survived longer than 10 days, the bile-duct epithelium had proliferated and lined partially necrotic ducts, with an associated increase in fibrous connective tissue in the portal areas. Frequently some bile ducts were occluded by tissue debris, inflammatory cells and proliferating fibrous tissue, producing an obliterative cholangitis (Fig. 5). Although not commonly observed, mineralization was present in some of the larger foci of hepatocellular necrosis.

Alterations similar to those in the intrahepatic bile ducts were observed within the extrahepatic ducts, with the additional occurrence of thrombosis of the

lymphatic vessels. The extrahepatic ducts passing within the pancreas were often necrotic, and necrotizing and inflammatory changes commonly extended into the adjacent pancreatic tissue. The severity of the secondary pancreatitis was related to the severity of the involvement of the extrahepatic ducts. Pancreatitis was observed in all groups, but was most severe in animals fed the fungal-mat diets.

Hepatocellular alterations were minimal and were similar for rats fed the rice-culture or fungal-mat diets. Infrequently, vacuolation of hepatocytes was observed, tending to occur more commonly in rats that had been anorectic for an extended period.

Renal lesions in rats fed the rice-culture diets were mild and consisted only of hyaline casts within the cortical tubules. Rats fed the fungal-mat diets showed

Table 2. *Macroscopic lesions observed in male rats fed a rice culture or fungal mat of* Aspergillus ochraceus

Test material and dietary level (%)	No. of rats affected per group of 12						
	Liver		Kidney	Scrotum		Stomach	
	Focal necrosis	Enlarged extrahepatic ducts	Green discoloration	Necrosis	Epididymal steatitis	Blood in lumen	Mucosal ulceration
Rice culture							
5	3	1	1	10	6	0	0
7.5	9	1	4	10	7	3	1
10	10	0	0	1	1	3	0
Fungal mat							
1	4	0	2	9	8	0	0
2	11	3	3	5	5	4	1
3	11	2	3	3	3	4	0

Table 3. *Microscopic hepatic lesions observed in male rats fed a rice culture or fungal mat of* Aspergillus ochraceus

Test material and dietary level (%)	No. of rats (per group of 12) affected by			
	Multifocal necrosis	Necrotizing cholangitis	Pericholangitis	Extrahepatic-duct necrosis
Rice culture				
0	0	0	0	0
5	10	10	11	2
7.5	10	10	10	4
10	9	10	8	6
Fungal mat				
0	0	0	0	0
1	4	10	10	5
2	12	12	12	10
3	11	12	12	9

Table 4. *Microscopic renal lesions observed in male rats fed a rice culture or fungal mat of* Aspergillus ochraceus

Test material and dietary level (%)	No. of rats (per group of 12) affected by		
	Tubular casts		Tubular necrosis
	Hyaline	Biliary	
Rice culture			
0	0	0	0
5	3	0	0
7.5	0	0	0
10	0	0	0
Fungal mat			
0	0	0	0
1	2	0	4
2	0	2	4
3	1	0	4

tubular degeneration and necrosis (Fig. 6), hyaline and biliary casts within tubular lumens and the presence of biliary pigment within the cytoplasm of renal tubules (Table 4). The glomeruli appeared normal.

Gastric lesions were found in rats fed the fungal-mat diets or the 5 or 7·5% rice-culture diets (Table 5). Gastric alterations were characterized by necrosis extending for about one third to two thirds of the depth of the mucosa. The craters contained necrotic leucocytes, erythrocytes, fibrin and tissue debris (Fig. 7). In the gastric submucosa, leucocytic infiltrates were accompanied by fibrinoid necrosis of small arteries and veins. No ulceration or necrosis was found in the intestine.

Scrotal lesions occurred in rats fed the fungal-mat diets or the 5 or 7·5% rice-culture diets. They were not found in rats fed 10% rice culture as all these died within 2 wk of the start of feeding and scrotal alterations did not become evident macroscopically until later in the feeding period. The lesions in the grossly swollen scrota were numerous foci of leucocytes and macrophages or a diffuse infiltration of mixed inflammatory cells within the epididymal connective tissue (Fig. 8). Later, the overlying epithelium became necrotic and the dermis and subcutaneous adipose connective tissue contained leucocytes and macrophages. Frequently, the walls of arterioles and venules had undergone fibrinoid necrosis (Fig. 9). In some rats fed for a longer period, medium to large foci of mainly mononuclear inflammatory cells were found within the epididymal fat, the principal site of involvement being the anterior epididymal adipose connective tissue. The adipose tissue was infiltrated mainly by leucocytes and macrophages arranged in either irregular foci, trabeculae or bands. Often, the foci of leucocytes were necrotic and mineralized. In severely affected rats, the inflammatory exudate extended dorsally and laterally within the scrotal fascia and into the fibrous connective tissue about the epididymal ducts. The epididymal ducts were often affected by the advancing necrotizing reaction, resulting in periductal inflammation, necrosis of duct walls and accumulation of inflammatory cells within the ductal lumina (Fig. 10). No changes were noted either in the testicular interstitium or in the seminiferous tubules.

Corneal oedema was observed in some rats of all the test groups (Table 5). Interstitial keratitis occurred only in some rats fed the 2 or 3% fungal-mat diets. Iritis occurred in one rat fed the 7·5% rice-culture diet and in two fed the 3% fungal-mat diet (Table 5). The earliest ocular change was oedema of the corneal stroma and hydropic degeneration of the corneal epithelium. The substantia propria was thickened and showed only pale staining, and the lamellae were disrupted and separated by oedemal fluid (Fig. 11). In some eyes, the basal layers of the corneal epithelium were separated by mild intercellular oedema, while in those more severely affected the substantia propria was diffusely infiltrated by neutrophils. Neocapillary vascularization was an infrequent finding. Iridocyclitis and hypopyon accompanied the acute interstitial keratitis in some eyes. The exudate in the anterior chamber consisted on neutrophils and mononuclear cells with formation of small keratic precipitates. Iridocyclitis was characterized by an accumulation of neutrophils within the stroma of the iris and ciliary body. In eyes with severe iridocyclitis, the iris was adherent to the lens capsule (Fig. 12).

DISCUSSION

Development of lesions in the liver of rats fed diets containing a rice culture or fungal mat of *A. ochraceus* followed a fairly definite pattern. Early, hypertrophy and hyperplasia of the bile-duct epithelium was accompanied by proliferation of the bile ducts. Later, a necrotizing cholangitis was associated with extension of the necrosis into the surrounding hepatic parenchyma, with periductal fibrosis and with stenosis and obliteration of the bile ducts. The hepatic lesions were identical to those produced in mice and rats by cultural products of *P. viridicatum* (Budiarso *et al.* 1971; Carlton *et al.* 1968 & 1970; McCracken, Carlton & Tuite, 1974c) and in mice by cultural products of *A. ochraceus* (Zimmermann *et al.* 1976). The morphological pattern of the hepatic lesions in rats and mice suggested that the lesion began in the

Table 5. *Microscopic lesions observed in the scrotum, eye, stomach and pancreas of rats fed a rice culture or fungal mat of* Aspergillus ochraceus

Test material and dietary level (%)	No. of rats affected per group of 12						
	Scrotum		Eye			Stomach	Pancreas
	Epididymal steatitis/ epididymitis	Epidermitis/ dermatitis	Iritis	Corneal oedema	Keratitis	Ulceration	Pancreatitis
Rice culture							
0	0	0	0	0	0	0	0
5	7	8	0	1	0	1	0
7.5	6	8	1	1	0	1	0
10	0	0	0	7	0	0	3
Fungal mat							
0	0	0	0	0	0	0	0
1	4	8	0	1	0	3	2
2	5	7	0	5	1	2	7
3	4	5	2	7	2	2	5

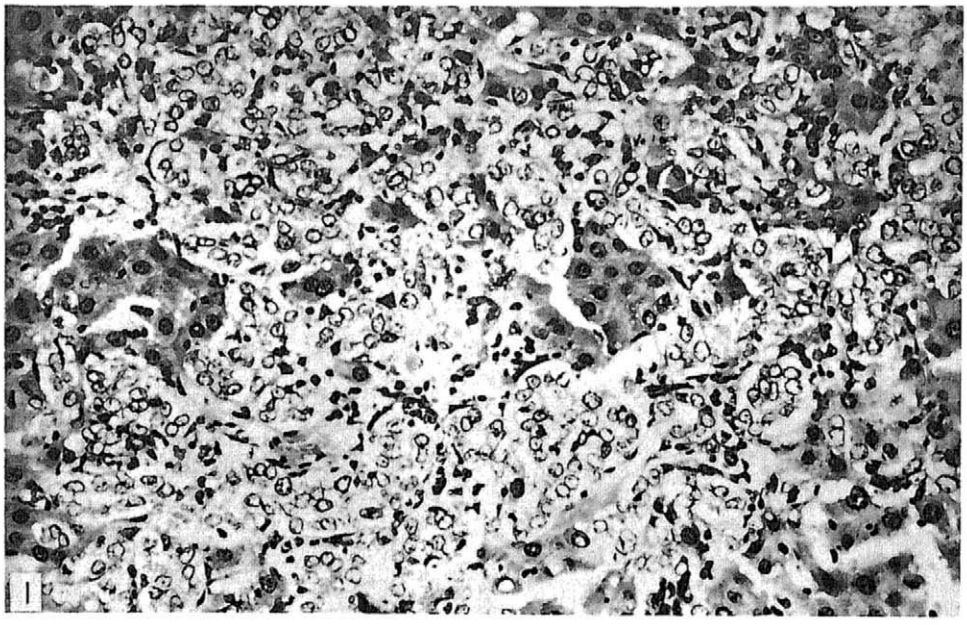

Fig. 1. Hyperplasia of biliary epithelium resulting in the formation of short columns and aggregates of cells. Haematoxylin and eosin (H/E) × 228.

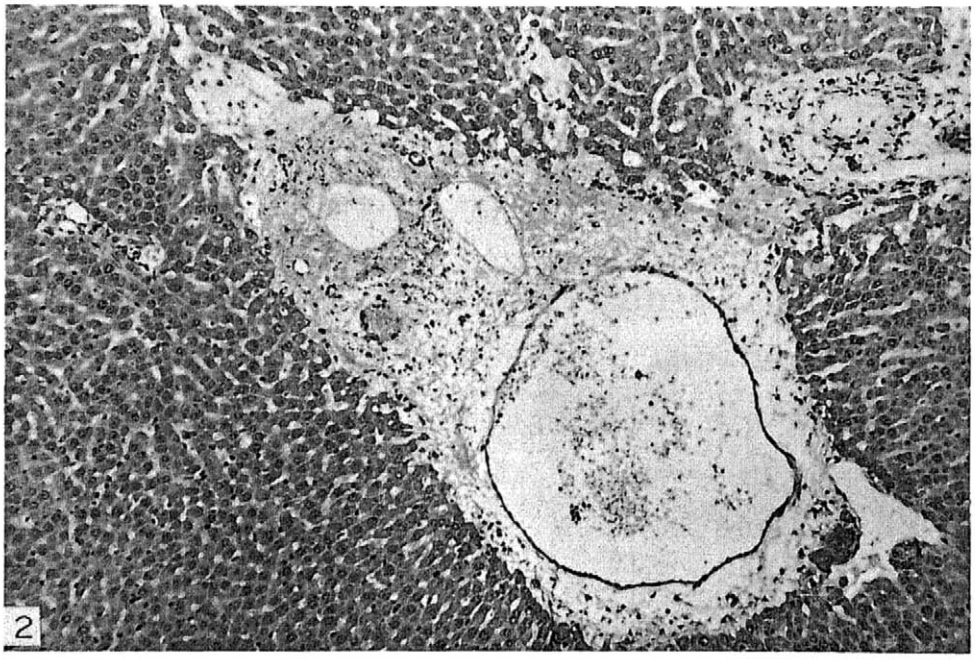

Fig. 2. Necrotizing cholangitis, characterized by necrosis centered about the portal triads. H/E × 88.

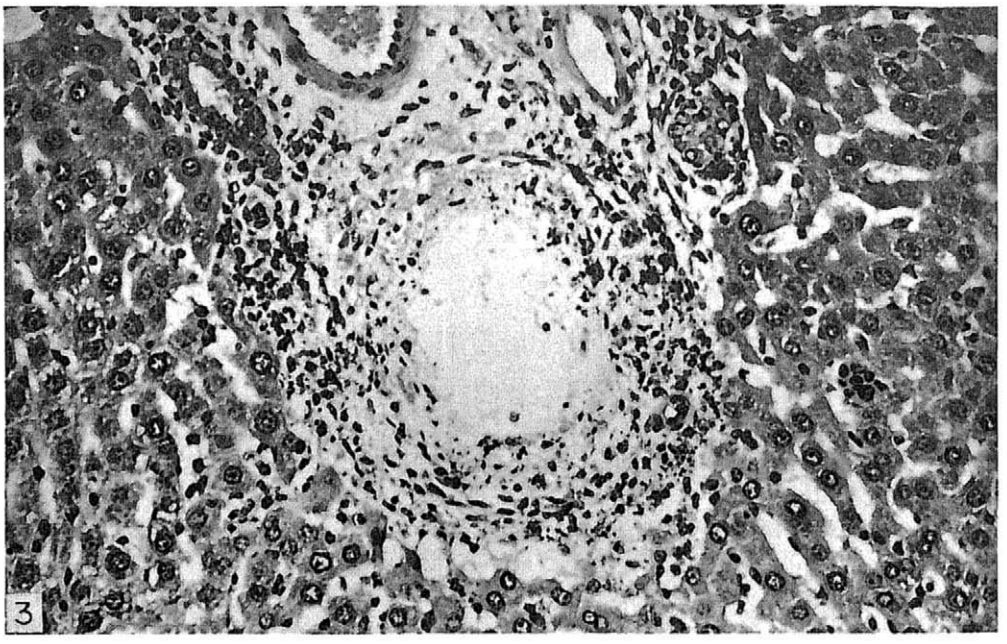

Fig. 3. Necrosis of intrahepatic bile ducts accompanied by periductal oedema and infiltration of the oedematous tissue with inflammatory cells. H/E × 228.

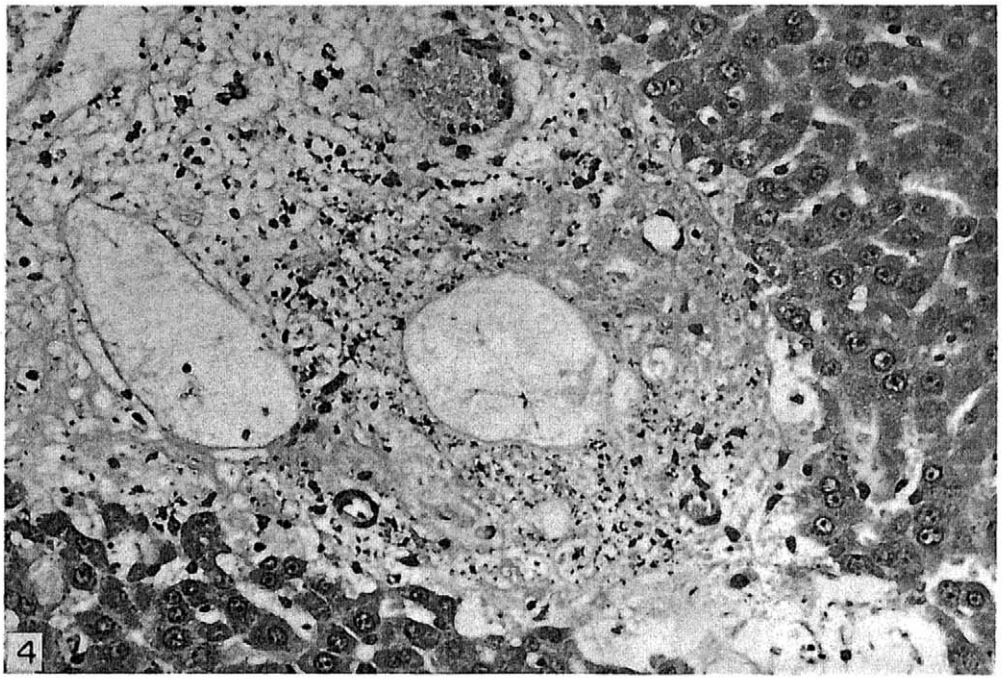

Fig. 4. Necrosis of portal structures, including bile ducts, the portal vein and hepatic artery and adjacent parenchyma. H/E × 228.

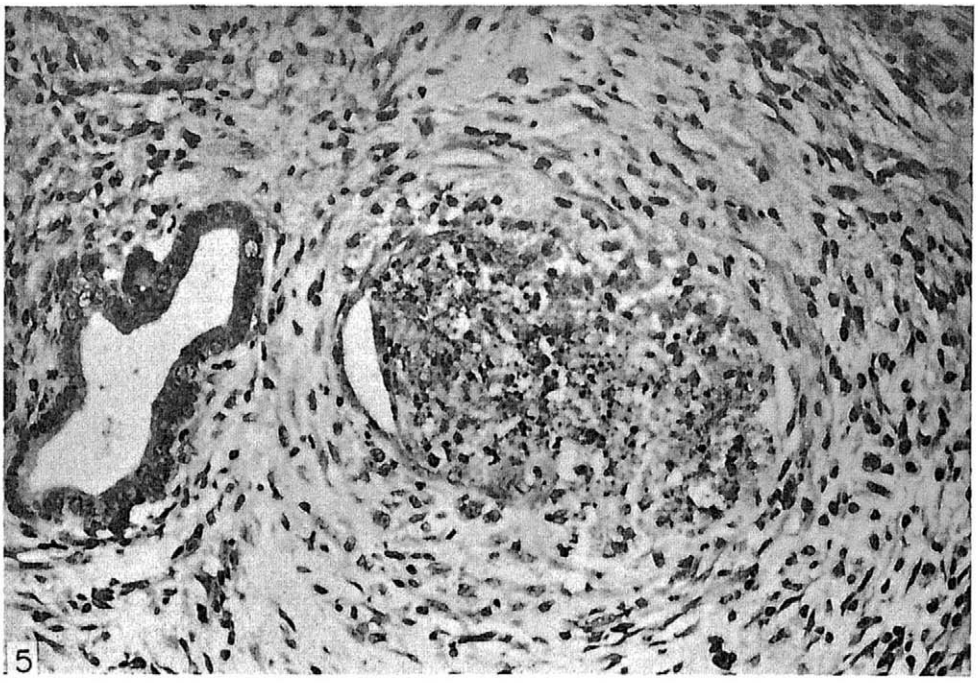

Fig. 5. Occlusion of bile duct by tissue debris and inflammatory cells, accompanied by periductular proliferation of fibrous connective tissue. H/E × 228.

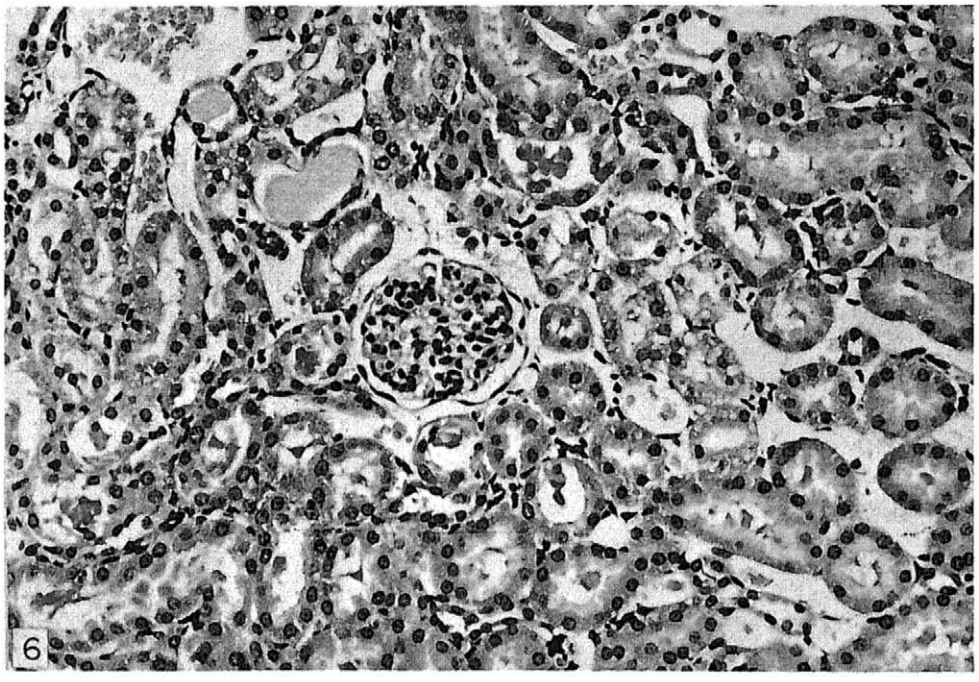

Fig. 6. Renal tubular degeneration with hyaline casts and a normal glomerulus. H/E × 228.

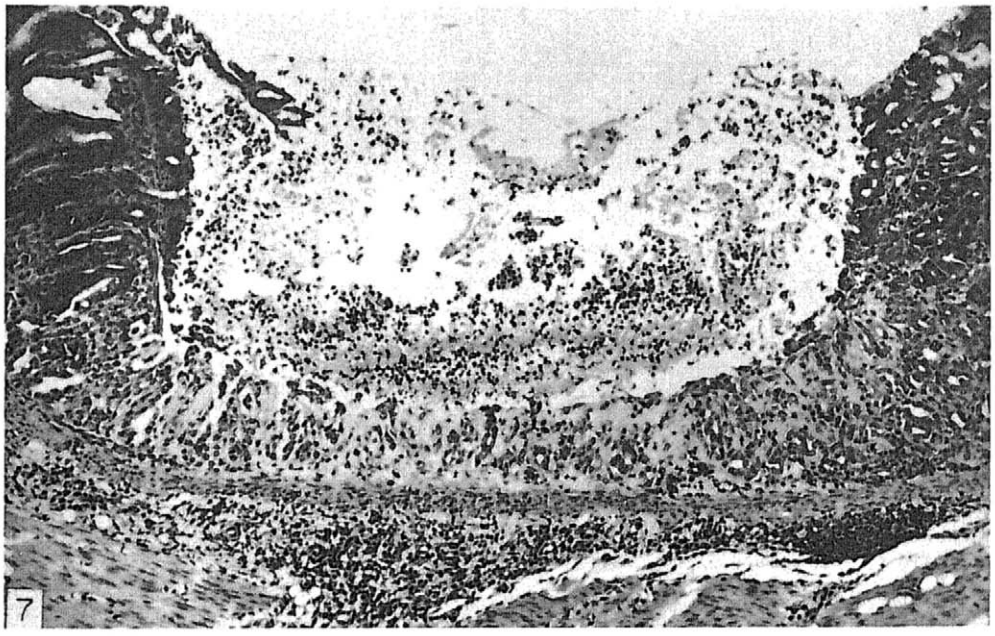

Fig. 7. Gastric erosion containing fibrin, tissue debris and necrotic epithelium, accompanied by submucosal cellulitis. H/E × 88.

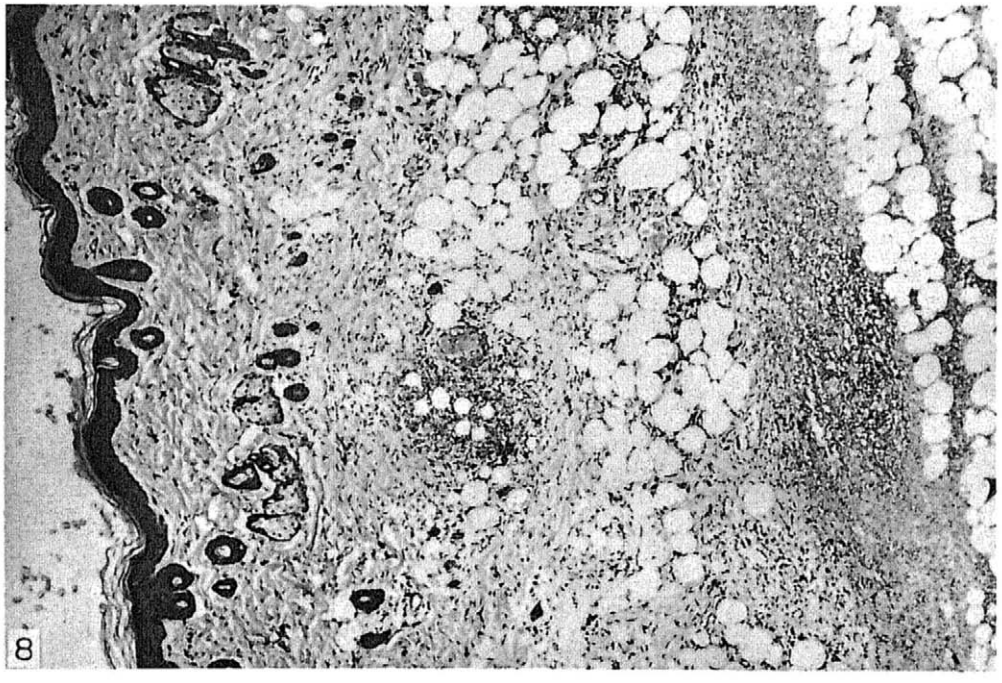

Fig. 8. Infiltration of inflammatory cells into the adipose tissue of the scrotal dermis. H/E × 56.

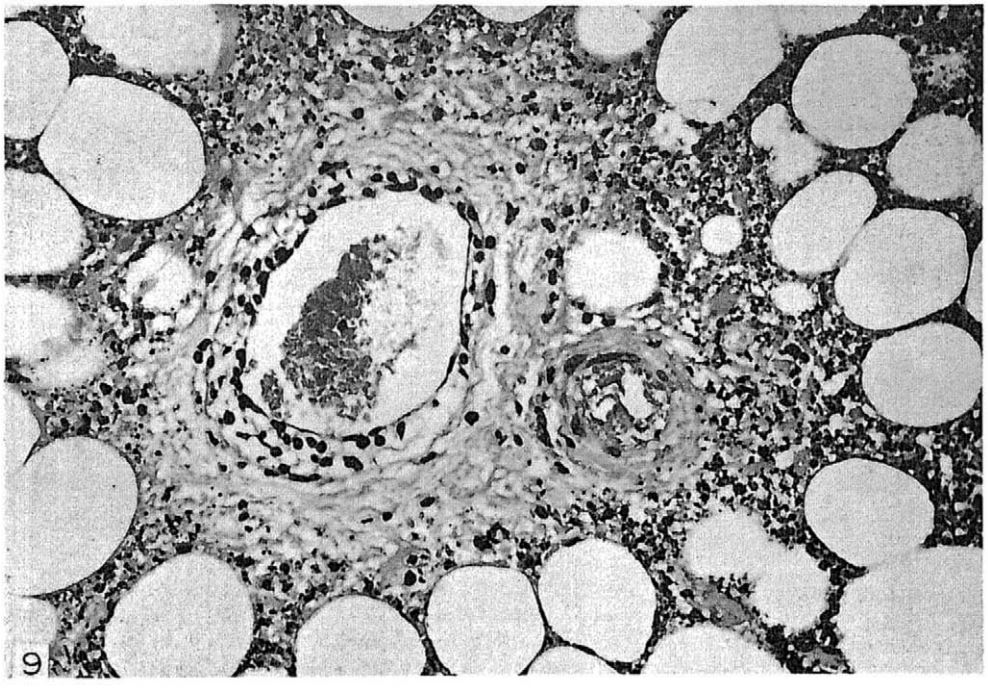

Fig. 9. Fibrinoid necrosis of an arteriole and phlebitis of an adjacent venule within the scrotal adipose connective tissue. H/E × 228.

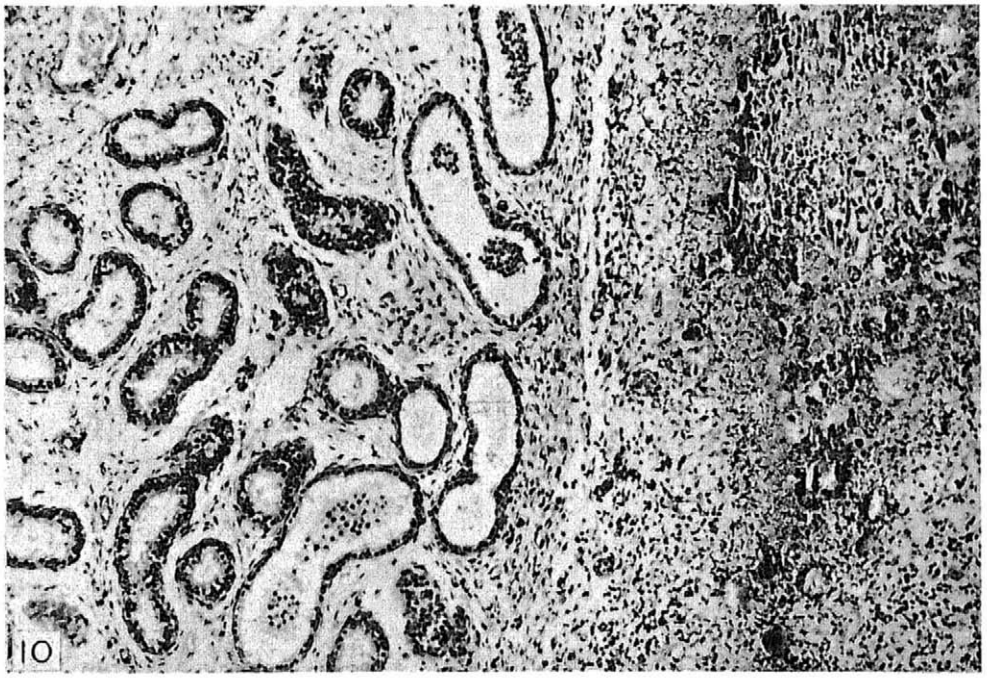

Fig. 10. Inflammation and necrosis of the epididymal adipose tissue, accompanied by an accumulation of inflammatory cells within the ductal lumina. H/E × 88.

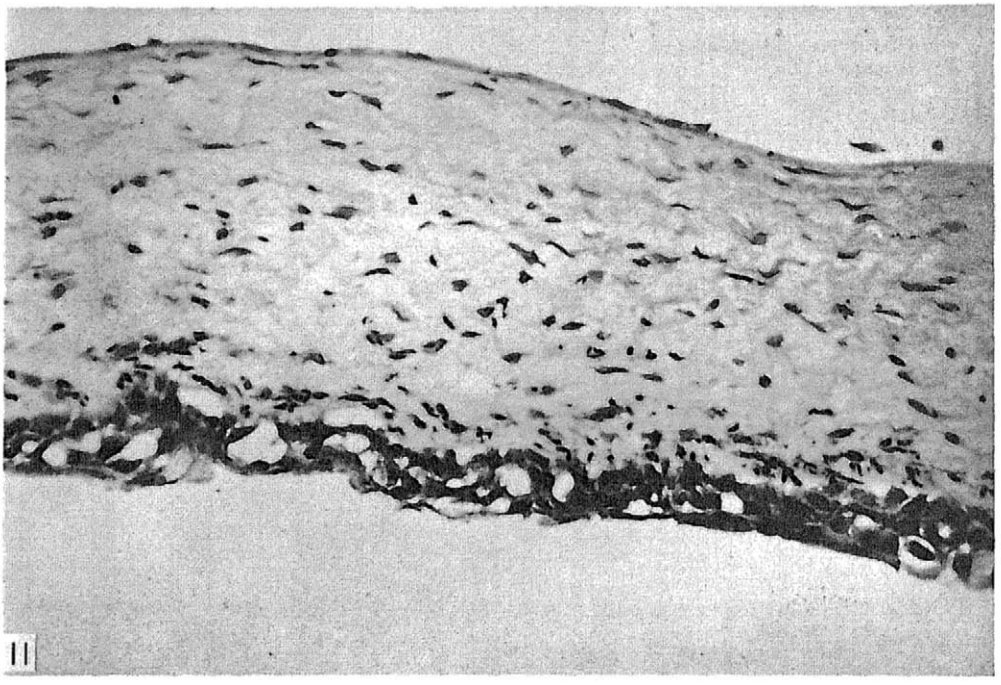

Fig. 11. Corneal oedema, hydropic degeneration of the epithelium and a mild infiltration of the substantia propria with a mixture of inflammatory cells. H/E × 228.

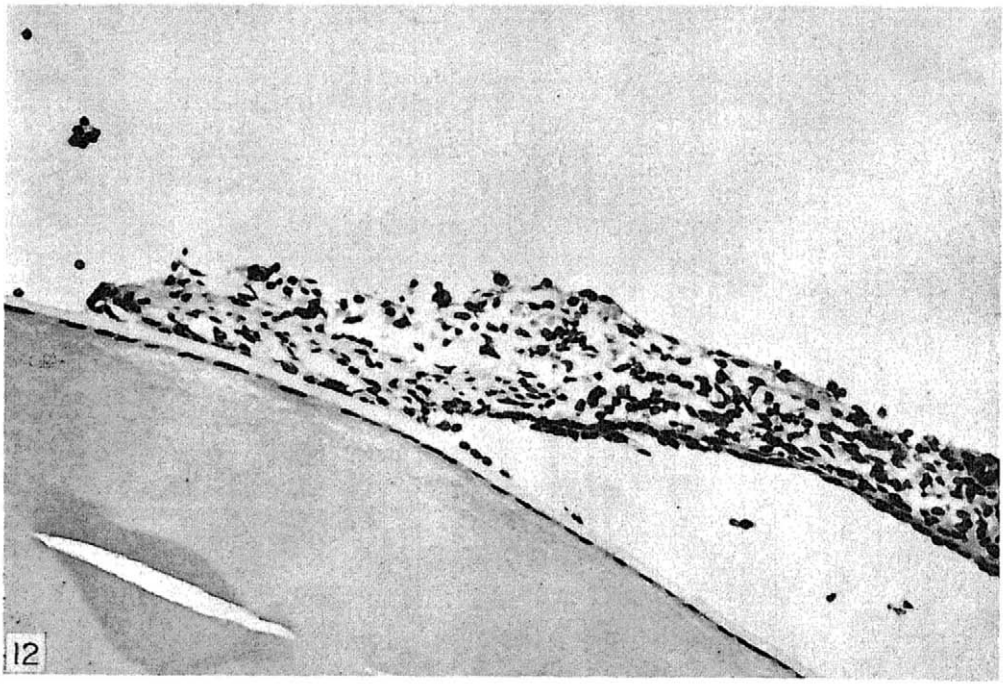

Fig. 12. An iris infiltrated with inflammatory cells and adhering to the anterior lens capsule. H/E × 228.

intrahepatic bile ducts and that the necrosis of the surrounding tissue was the result of the damage to the biliary system, allowing leakage of bile or toxin(s) into the surrounding hepatic parenchyma.

The hepatic lesions observed in these rats showed some similarity to lesions induced in rats by sporidesmin (Remington, Slater, Spector, Strauli & Willoughby, 1962). However, the pleural effusions and ascites that were prominent findings in sporidesmin poisoning were not seen in the rats of this study. Other hepatotoxic mycotoxins do not produce a necrotizing cholangitis or cholangiohepatitis and most are characterized by hepatic degeneration and necrosis, sometimes accompaneid by bile-duct hyperplasia. Such mycotoxins include the aflatoxins (Wogan, 1965), luteoskyrin (Kobayashi, Uraguchi, Sakai, Tatsuno, Tsukioka, Sakai, Sato, Miyake, Saito, Enomoto, Shikata & Ishiko, 1958; Uraguchi, Tatsuno, Sakai, Tsukioka, Sakai, Yonemitsu, Ito, Miyake, Saito, Enomoto, Shikata & Ishiko, 1961), cyclopiazonic acid (Purchase, 1971), the epoxytrichothecenes (Saito, Enomoto & Tatsuno, 1969) and rubratoxin A produced by certain strains of *P. rubrum* (Wogan, Edwards & Newberne, 1971). Ochratoxin A toxicosis results in non-specific hepatocellular degeneration and necrosis. However, this particular mycotoxin has not been found in our isolate of *A. ochraceus*. Several species of Aspergilli can produce sterigmatocystin, which causes focal hepatic necrosis, proliferation of bile ducts, pericholangitis and hyperplastic nodules (Purchase & van der Watt, 1969). Of the mycotoxins, then, only the sporidesmins (and the pigments xanthomegnin and viomellein) elicit tissue responses similar to those observed with *A. ochraceus* and *P. viridicatum*. Metabolites with chemical characteristics consistent with the sporidesmins have not been isolated from cultures of *A. ochraceus* or *P. viridicatum*.

Gastric ulceration and varying degrees of submucosal cellulitis were present in some of our rats. Necrosis of the epithelium of the digestive tract and, in particular, of the crypt epithelium has been observed in rats and mice given extracts of *Fusarium nivale* (Tatsuno, 1968) and with the purified mycotoxins nivalenol (Tatsuno, Saito, Enomoto & Tsunoda, 1968) and fusarenon-X (Ueno, Ueno, Iitoi, Tsunoda, Enomoto & Ohtsubo, 1971). Ulceration of the intestinal mucosa and necrosis of the crypt epithelium were not observed in our rats fed *A. ochraceus*.

Scrotal lesions similar to those observed in our rats fed cultural products of *A. ochraceus* have been described in rats given cultural products of isolates of *P. viridicatum* (Carlton & Tuite, 1970; McCracken, Carlton & Tuite, 1974b; Rafiquzzaman, 1974). Budiarso, Carlton & Tuite (1970) described testicular lesions in mice fed *P. viridicatum*. No set of similar scrotal lesions has been reported in the rat as a result of either an infection or a mycotoxicosis. Scrotal lesions in our rats began and were most severe and numerous in the epididymal fat. At an early stage, mononuclear cells infiltrated the dermis and the epididymal fat and these infiltrates formed discrete accumulations of leucocytes and macrophages frequently oriented about blood vessels. Later, bands of necrotic leucocytes and macrophages transsected the junction between the dermis and subcutis with obliteration of large portions of the epididymal adipose connective tissue. The epidermis was not consistently involved and most often seemed to be secondarily affected at a late stage of the cellulitis. The development of lesions deep within the scrotal tissue, many centering about blood vessels, and the lack of concomitant involvement of the epidermis indicated that the changes were the result of circulating toxin(s) and not due to surface contamination with toxic diet. Several of the trichothecenes, such as diacetoxyscirpenol, T-2 toxin, HT-2 toxin, nivalenol and fusarium-X produced by several species of Fusarium and other fungal genera, are highly toxic when applied topically to the skin of rats, mice and rabbits, but cutaneous lesions have not been described following oral or parenteral administration (Bamburg & Strong, 1971).

The cutaneous scrotal lesions in our rats were initially multiple foci of inflammation and necrosis within the subcutis, progressing subsequently to epidermal ulceration. McCracken *et al.* (1974b) described similar alterations in rats fed cultural products of *P. viridicatum* and suggested that the lesions within the scrotal adipose connective tissue were due to some blood-borne toxin(s). By sequential killing of the rats, McCracken *et al.* (1974b) demonstrated a definite association between inflammatory cell infiltration about blood vessels, vascular thrombosis and epidermal necrosis. In our rats, the epithelium of the epididymal ducts and seminiferous tubules was not primarily altered, but testicular lesions have been reported with a number of other mycotoxins, including extracts of *F. nivale*, nivalenol (Ueno *et al.* 1971) and neosolanil (Ishii, Sakai, Ueno, Tsunoda & Enomoto, 1971; Ueno, Ishii, Sakai, Kanaeda, Tsunoda, Tanaka & Enomoto, 1972). The necrotizing scrotal lesions appear to be a unique response of rats to cultural products of *A. ochraceus* and *P. viridicatum* as they were not found in guinea-pigs (Carlton & Tuite, 1970) or mice (Budiarso *et al.* 1970) fed *P. viridicatum*, and have not been described in other mycotoxic diseases including those caused by aflatoxin (Wogan, 1965), ochratoxin A (Purchase & Theron, 1968), rubratoxin (Wogan *et al.* 1971) and sterigmatocystin (van der Watt & Purchase, 1970).

Ocular lesions developed in most rats fed rice-culture or fungal-mat diets. The earliest alteration was corneal oedema and this was often followed by interstitial keratitis and hypopyon. Because normal hydration and transparency of the corneal stroma is dependent upon the balance between the influx of fluid into and the active transport of fluid out of the stroma (Dikstein & Maurice, 1972; Mishima & Hayakawa, 1972) and because the control mechanism for this resides in the corneal endothelium (Dikstein & Maurice, 1972; Mishima & Hayakawa, 1972), it seems possible that circulating toxin(s) might be present in the anterior chamber, resulting in damage to the corneal endothelium. McCracken, Carlton & Tuite (1974a) described similar ocular lesions in rats fed cultures of *P. viridicatum*. Several fungal extracts and mycotoxins have produced corneal opacity when applied topically into the conjunctival sac of rabbits. These include purified sporidesmin and extracts of *Pithomyces chartarum* (Done, Mortimer, Taylor & Russell, 1961), patulin (Broom, Bulbring, Chapman,

Hampton, Thomson, Ungar, Wein & Woolfe, 1944) and diacetoxyscirpenol (Brian, Dawkins, Grove, Hemming, Lowe & Norris, 1961), but none of these are known to produce ocular lesions following oral or parenteral administration.

Bacteria were not detected in swabs from the interior of the eyes of rats fed the rice culture or fungal mat. Two rats had inflammatory changes of the Harderian gland consistent with the changes described for sialodacryoadenitis of rats caused by a corona virus (Hunt, 1963; Jacoby, Bhatt & Jonas, 1975; Jonas, Craft, Black, Bhatt & Hilding, 1969). These Harderian gland lesions were incidental and occurred in rats with and without lesions of the cornea and anterior uvea.

A toxin has not been recovered in pure form from our strain of *A. ochraceus*. Analyses carried out by one of us (J.T.) in the Department of Botany and Plant Pathology have shown the cultures to be negative for aflatoxin, ochratoxin A, citrinin and penicillic acid. Stack, Eppley, Dreifuss & Pohland (1977) isolated viomellein and xanthomegnin pigments from an Indiana isolate of *P. viridicatum*, which produced hepatic lesions in mice and rats identical to those induced by our strain of *A. ochraceus*. These pigments, xanthomegnin and viomellein, incorporated into a purified diet and fed to mice, produced hepatic lesions identical to those produced by cultural products of *P. viridicatum* (Carlton, Stack & Eppley, 1976). Experiments are currently being conducted to determine whether these pigments and others are also present in cultural products of *A. ochraceus*.

Acknowledgements—This research, published as Journal paper no. 7144 of the Agricultural Experiment Station, Purdue University, was supported in part by NIH Grant ESCA-00463. John L. Zimmermann is an Eli Lilly Postdoctoral Fellow.

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