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EXPERIMENTAL *PHOMOPSIS LEPTOSTROMIFORMIS* MYCOTOXICOSIS OF PIGS

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SUMMARY

The susceptibility of the domestic pig to intoxication by the causative mycotoxin of lupinosis was established experimentally. The symptomatology and pathology of the disease produced by the administration of toxic cultures of the fungus, *Phomopsis leptostromiformis* (Kühn) Bubák ex Lind to pigs are described. The toxin induced severe loss of weight and, in many cases, posterior paresis or paralysis. The principal gross lesions were generalised icterus, orange-red discoloration of the liver, nephrosis and, in some, enterorrhagia. Microscopically there was severe necrosis of hepatocytes and kidney tubular epithelium as well as myocardial degeneration. In the more chronic cases hepatocytes became anaplastic and arranged in acini. The production of toxin by *P. leptostromiformis* on yellow maize is reported and a method for production of toxic material is described.

INTRODUCTION

It has now been well established that lupinosis is a mycotoxicosis caused by the fungus *Phomopsis leptostromiformis* (Kühn) Bubák ex Lind (= *Phomopsis rossiana* (Sacc.) Sacc. et D. Sacc.[7 10 18 19 20]). Its morphology and synonomy have been described by Van Warmelo & Marasas[17]. The mycotoxin responsible for the disease is also produced on substrates other than lupin material[20].

Lupinosis has been described in various species namely sheep[1 4 5 6 18 19], cattle[12], horse[8] and three species of experimental laboratory animals[3 4 7 10 13 14]. Only one brief report exists in the literature on a possible field outbreak of lupinosis in swine[11]. In this instance pigs fed a ration containing ground, bitter lupin seeds suddenly became ill and showed signs of inappetence, depression, recumbency, increase in body temperature, slight icterus of the sclera, constipation, vomiting and lack of milk production.

Because there appears to be a tendency to make more use of lupins in pig rations due to the increase in price of soybeans[9] it was decided to investigate the susceptibility of pigs to the mycotoxin produced by the fungus, *P. leptostromiformis*.

MATERIALS AND METHODS

In our laboratory over the past 4 years we have used for experimental purposes the isolate of *P. leptostromiformis* described as No. B16 (= PRE 44350). It was isolated from pods of *Lupinus albus* L. cult, Pflugs Gela, which were obtained from the Hermon district, Cape Province in October 1969[17 18 19]. Subcultures of this isolate have been deposited in the American Type Culture Collection (ATCC 22849), Centraalbureau voor Schimmulcultures (CBS 754.70) and the Commonwealth Mycological Institute (IMI 146035). Stock cultures were maintained on slants of 1,5% malt extract agar in McCartney bottles at 4°C. As we have determined that pure cultures of the fungus grown on autoclaved maize (*Zea mays* L.) kernels induced lesions in sheep, rabbits, guinea pigs and mice identical to those caused by cultures on *L. albus* seeds (Marasas, Kellerman, Anderson and Van Rensburg 1971, unpublished data), and since maize was more easily obtained we adopted it as a standard medium for production of toxic material. In this experiment the procedure was as follows: Spore suspensions of *P. leptostromiformis* for use as inoculum were prepared in sterile water from 21 day-old sporulating cultures grown on 1,5% malt extract agar[17 18].

Yellow maize kernels in distilled water were autoclaved for 1 h on each of 2 consecutive days at 121°C and 103 k Pa in 1,0 *l* glass fruit jars (200 g kernels in 150 ml distilled water/jar). The autoclaved kernels in each jar were inoculated with 2,5 mℓ of spore suspension. The jars were shaken to distribute the spores evenly and incubated in the dark at 25 to 28°C for 4 weeks. Thereafter the contents of the jars were minced in a meat mincer, air-dried at room temperature in flat metal pans, ground to a fine powder in a mill and stored in a refrigerator at 4°C until used.

DOSING OF FUNGAL CULTURES TO EXPERIMENTAL PIGS

PILOT EXPERIMENT

A pilot experiment using one pig was done in 1971. A Large White gilt about 4 months of age received *Phomopsis* culture plus maize medium mixed with her normal pig growing ration at 5 g/kg body weight for 2 consecutive days. This resulted in anorexia and apathy for several days after which the animal recovered. When she had fully recovered her appetite, toxic material was again mixed into her feed. This was repeated for approximately 8 weeks by which time a total of approximately 950 g of toxic culture and medium had been consumed. She died and was autopsied on the 58th day of the trial. A litter mate kept as a control was fed only the pig growing ration.

EXPERIMENT I

Six Landrace X Large White pigs, 2 months old and representing both sexes were purchased from a

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breeder and fed *ad lib.* on a commercial balanced ration known as " pig growth meal*". They were allowed 2 weeks to adapt to their new surroundings and feed. Pig No. 1 was starved for 24 hours and then given toxic material (fungus plus substrate) at the rate of 20 g/kg body weight mixed with a sufficient quantity of its ration for it to consume in a few minutes. The same procedure was followed with Pig No. 2, but it received 10 g/kg body weight of toxic material. Both pigs consumed the full dosage. Pig No. 2 was again offered 10 g/kg 4 days later, but only a few mouthfulls were eaten. Pigs No. 3 and 4 were each given a single dose of 20 g/kg body weight of the toxic material in a similar way while Nos. 5 and 6 only received the normal ration and served as controls. The animals were weighed every week. Pigs surviving the experimental period of 2 weeks were slaughtered by stunning and exsanguination. All pigs were autopsied and material collected from them for histopathological examination.

EXPERIMENT II

Because the toxin causes complete anorexia in pigs for several days following its administration repeated administration of toxic material at short intervals by the method described above is impossible. The dosing of pigs with large amounts of any material is also virtually impossible. Therefore intragastric rubber fistulas** were installed in eight pigs according to the method described by Michael & Buck[12]. These animals were of the same mixed breed and of both sexes as those in Experiment I and at the time of purchase weighed approximately 35 kg (see Table 1). The surgery was performed under Trilene*** anaesthesia after premedication with Stresnil.****

The pigs were allowed 1 week to adapt to the fistulas. Toxic material was then administered to the experimental animals and autoclaved minced and dried yellow maize kernels to the controls through the fistulas directly into the stomach as follows: The required quantity was weighed and mixed with tap water (the same as was available in their drinking troughs) into a semifluid mass and then pumped through the fistulas by means of a stomach pump. The pigs did not object to this. Dosage rates were as follows: Pigs F1 and F2 received 10g toxic material/kg body weight daily on 5 consecutive days of the week until death supervened. Feed and water were withheld for 16 h prior to administering the material. Pigs F3 and F4 were given 2,5 g toxic material/kg body weight on 3 days of the week, *i.e.* Mondays, Wednesdays and Fridays. Toxic material was administered at the rate of 1,25 g/kg body weight to Pig F5 once a week only and to F6 fortnightly for the first month and thereafter once a week. The control pigs F7 and F8 received 5 g/kg body weight of the yellow maize kernels on Mondays and Fridays during the experiment. The pigs were examined daily and weighed at weekly intervals.

Pigs surviving the experimental period of 8 weeks and those very sick or *in extremis* were stunned and exsanguinated. All animals were autopsied and specimens from various organs were taken and fixed in 10% buffered formalin. Blocks prepared from these were embedded in paraffin wax. Sections were then cut and stained with haematoxylin and eosin (H & E) in a routine manner for microscopical examination.

RESULTS

Some of the experimental data and results are summarized in Table 1.

PILOT EXPERIMENT

CLINICAL AND GROSS PATHOLOGY

The experimental animal was at the time of death in a very poor physical condition and her weight was

* Epol Feeds (Pretoria) (Pty.) Ltd.
** Hudson Vulcanising Company.
*** I.C.I. South Africa (Pharmaceuticals) Ltd.
**** Ethnor Laboratories (Pty.) Ltd.

Table 1: SUMMARY OF EXPERIMENTAL DATA

Experiment	Pig		Weight in kg			Duration of experiment in days and manner of death	Total amount of toxic material received in g	Number of doses
	No.	Sex	Commencing	Death	Gain or loss			
Pilot		f	28	25	−3	58D	950	8
I	1	f	26	24,5	−1,5	7E	520	1
	2	f	26	22,5	−3,5	13E	260+x	2
	3	m	30	–	–	3K	600	1
	4	m	34,5	33	−1,5	5K	690	1
II	F1	m	36	32	−4	12E	3 550	10
	F2	f	39	38	−1	7D	1 950	5
	F3	f	40	28	−12	19E	740	9
	F4	f	41	30	−11	15D	605	6
	F5	f	36	16	−20	42D	200	7
	F6	m	41	36	−5	46E	296	6
	F7	m	41	47	+7	7K	0	0
	F8	f	41	80	+39	56K	0	0

f = female m = castrated male K = killed E = killed in extremis or very ill D = died x = estimated 80g eaten at 2nd feeding.

less than half that of the control animal (*i.e.* 25 kg compared with 69 kg). A generalised icterus was present and the skin was congested and cyanotic. The liver was of normal size, a yellow-brown colour and of increased consistency. The bile was a curry yellow, turbid and floccular fluid. Subcutaneous and intermuscular oedema was conspicuous – especially in the ventral neck region. The large intestine contained hard, dry faecal balls coated with mucous and blood. Hydropericardium, pulmonary oedema and sub-epi-, and endocardial haemorrhages were present.

HISTOPATHOLOGY

The lesions in this animal were of a more chronic nature than any of the subsequent cases. The liver presented the most severe changes. The basic lobular architecture was well preserved but within the lobules, however, hardly any hepatocytes were present. There was no orderly cord-like arrangement of parenchymal cells. The majority of hepatocytes had been replaced by small anaplastic cells showing a tendency to acinar arrangement. Many of these cells contained small lipid vacuoles. What few hepatocytes were left contained numerous small fat droplets in their cytoplasm and were frequently multinuclear. Yellow pigment globules were present in some of them. The nuclei varied in size and shape, some were very small and pycnotic, others were large and bizarre in that they had a multiplicity of shapes while a few cells contained larger than normal round nuclei. Cells containing the latter occurred most frequently at the periphery of the lobule. The centres of the lobules were congested and haemorrhagic. There was some intralobular round cell and fibroblast infiltration and proliferation.

Several myocardial fibres contained small clear vacuoles while in others the nuclei were enlarged and some proliferation of sarcolemma nuclei had occurred. In the left papillary muscle there was a rarifaction of the myocardium with homogenous eosinophilic clumping of sarcoplasm in these areas. Some fibres were calcified (Fig. 3).

EXPERIMENT I

CLINICAL AND GROSS PATHOLOGY

Pig 1 weighed 26 kg on the day the experiment commenced and 24,5 kg when it was killed *in extremis* on the 7th day. From the third day it was anorectic, very lethargic and showed a very slight yellowish tinge to the sclera.

When necropsied the most interesting finding was a slightly smaller than normal orange-brown liver with a definite increase in consistency. The bile was of a normal quantity but mucoid and dark-yellow. The intima of the large blood vessels was a light yellow colour and the kidney cortex pale brown and streaky in appearance on cut surface indicating mild degenerative changes.

Pig 2 had the same initial weight and was killed 13 days later for necropsy at which time it had lost 3½ kg body weight. The clinical signs exhibited were identical to those of Pig 1.

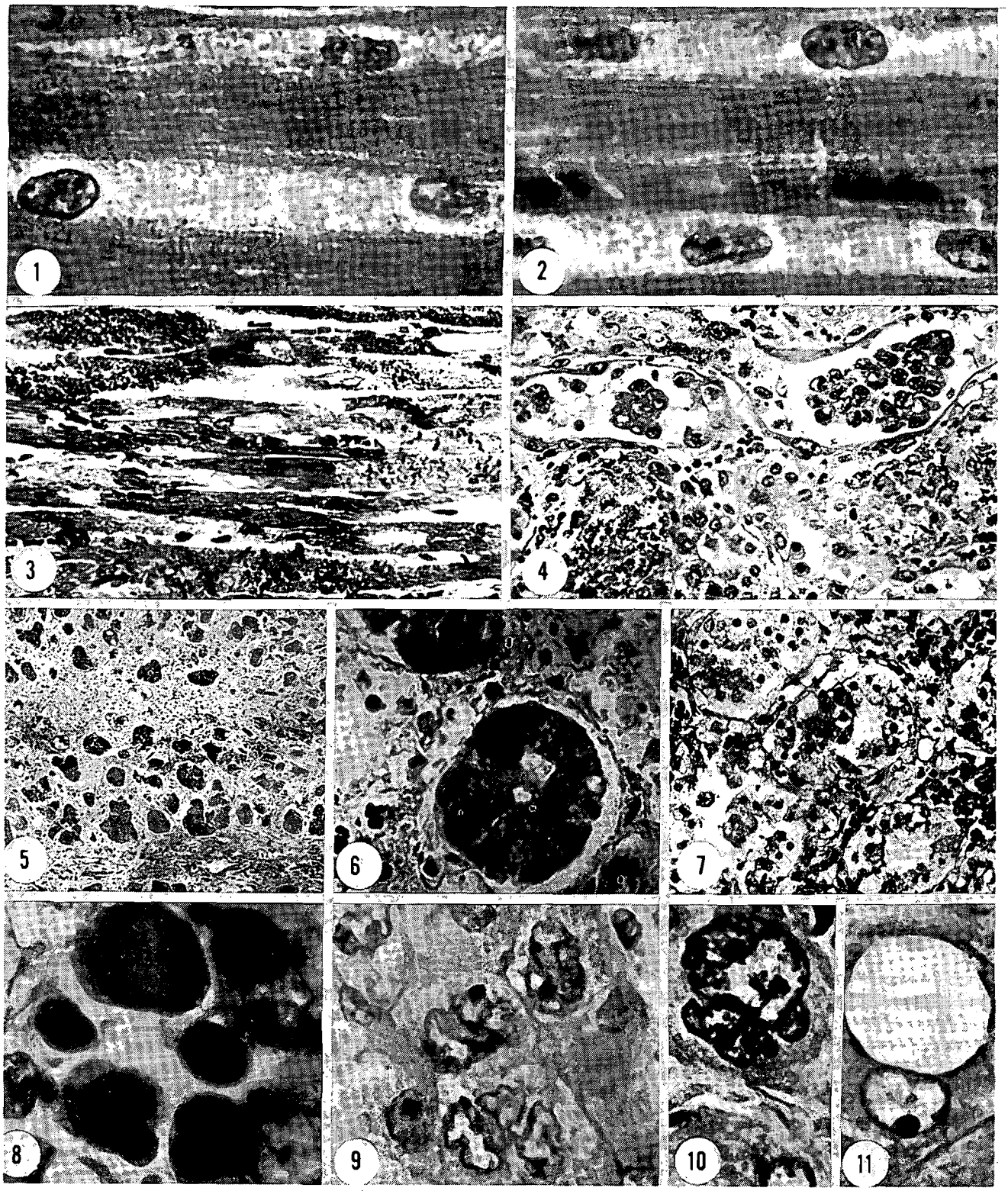
Macroscopic lesions were a slight icterus, an enlarged orange-yellow liver which was firmer than normal in consistency, but not as firm as in the case of the former pig. The bile was a light green (resembling unripe grapes) and of jelly-like consistency. The kidneys were enlarged and a light biscuit-brown colour.

Pig 3 was killed for necropsy on the third day post toxin administration. Macroscopically the liver was a slightly paler brown than normal with bile resembling that of the former pig.

Pig 4 weighed 34½ kg before feeding of toxic material and was killed on Day 5 after showing anorexia for 2 days and having lost 1½ kg of body weight. Macroscopically there was a conspicuous icterus best observed in the intima of the large blood vessels. The lymph and joint fluid were a picric acid yellow. The liver had a firmer consistency and the lobulation was more distinct than normal with the lobules having a brown central portion and greyish periphery. The bile was a clear dark yellow colour with an orange granular deposit. The kidneys were a light fawn. Despite the anorexia clinically the stomach was well filled with food on examination.

HISTOPATHOLOGY

The histopathological changes of the livers of Pigs 1 and 2 were very similar — the difference being mainly a matter of degree. The lobular architecture was not disturbed although the parenchyma within the lobules was disrupted. Focal areas of leukocytic infiltration occurred in the portal areas as well as in the lobules especially in Pig 2. Fatty changes of the hepatocytes were very mild and bile duct proliferation within the lobule was present but inconspicuous. The liver of Pig 1 especially showed an increase of collagen and fibroplasia in the portal areas. These were accompanied by some bile duct epithelial cell hyperplasia. The normal cord-like arrangement of hepatocytes was disturbed, the sinusoids being compressed by encroaching hepatocytes in many instances. There was a marked variation in the degree in which hepatocytes stained with H & E. The cytoplasm of some was very granular and light pink, in others it was intensely and homogenously eosinophilic or it contained basophilic blotches. The nuclei presented even a greater variation. Many cells were binucleate or multi-nucleate with up to six nuclei per cell. The nuclei differed markedly in size, even in the same hepatocyte. Most nuclei were round with a distinct basophilic nuclear membrane, vesicular nucleoplasm and one or more distinct nucleoli. Some had large, bizarre shapes and gave the impression of "budding" as described by Petterson & Coackly[15] while others contained distinct pink pseudo-inclusions which resulted from cytoplasmic invagination. Some nuclei on the other hand were pyknotic and undergoing karyorrhexis. A moderate number of hepatocytes contained round, discrete or multiple, small eosinophilic globules in the cytoplasm while in others there was a large eosinophilic mass lying in a vacuole — in some cases containing pyknotic nuclear remnants. In general the centrilobular hepatocytes were smaller than the more peripherally situated ones. Among these central cells were some which contained conglomerates of a nongranular brown pigment



Figs. 1&2 Myocardial degeneration in Pig 3. H&E X 1200

Fig. 3 Myocardial degeneration and calcification in the pig used in the pilot experiment. H&E X 250

Fig. 4 Kidney of Pig F4 showing necrosis and desquamation of tubular epithelium with cast formation. H&E X 250

Fig. 5 Liver of Pig F6 showing grouping of hepatocytes into acinar structures. H&E X 80

Fig. 6 Higher magnification of Fig. 5. H&E X 250

Fig. 7 Liver of the pig used in the pilot experiment showing acinar arrangement of hepatocytes. H&E X 250

Fig. 8 Large eosinophilic globules in hepatocytic cytoplasm of Pig F3. H&E X 1200

Fig. 9 Crenated nuclei with nuclear division in Pig F5. H&E X 1200

Fig. 10 Large "budding" nucleus from the kidney of Pig F4. H&E X 1200

Fig. 11 Binucleate hepatocyte with one large vesicular nucleus in Pig F3. H&E X 1200

resembling bile in bile canaliculi in the cytoplasm. This pigment was negative for stainable iron. Its nature could not be determined.

The kidney of Pig 1 revealed a large percentage of cells with mitotic figures in the tubular epithelium.

The histopathological changes in the liver of Pig 3 were similar to those of the foregoing, but were not quite as extensive. There was no increase in connective tissue, and the nuclear changes were mostly necrotic in nature, namely pyknosis and karyorrhexis — the latter frequently resembling mitotic figures. In the kidney several epithelial cells in the convoluted tubules exhibited mitoses. The cytoplasm of the majority of these cells was swollen and had a granular appearance. Under high magnification small clear intracytoplasmic clefts could be determined in many of them. This was interpreted as being a sign of early hydropic degeneration. In the myocardium were numerous focal areas involving groups of muscle fibres where small eosinophilic droplets had formed around and in the vicinity of the nuclei (Figs. 1 & 2).

In the liver of Pig 4 small aggregations of monocytic cells and a few neutrophiles were seen in some lobules. The centrilobular cells were atrophic. A degree of leukostasis also occurred in the central zone. The most conspicuous change occurred in the peripheral areas. Numerous hepatocytes had very pyknotic nuclei and a cytoplasm which was condensed and eosinophilic around the nucleus but clearer at the periphery of the cell. Some, however, were frankly karyorrhectic. The nuclei of some liver cells were enlarged and bizzare in shape and many cells were binucleate or even multinucleate. The nuclei in these cells were mostly of normal size while in some a very clear margin and large distinct nucleolus was the only abnormality seen. Several hepatocytes contained distinct single, golden-brown pigment globules in their cytoplasm while in others rounded-off masses of eosinophilic cytoplasm were present, some containing nuclear debris. Fatty change was not observed but there was a slight increase in fibrous connective tissue between lobules, as well as in bile duct epithelial cells and bile ducts in some portal areas. The latter was not conspicuous.

The kidney in this case was severely affected: the majority of epithelial cells in the convoluted tubules showing granular eosinophilic cytoplasm with pyknotic or karyorrhectic nuclei. A few cells contained, as in the liver, rounded masses of necrotic cytoplasmic and nuclear debris in their cytoplasm. In many areas necrotic cells had desquamated and been replaced by regenerating epithelial cells.

The two pigs kept as controls were normal in all respects.

EXPERIMENT II

CLINICAL OBSERVATIONS

Of the two pigs receiving 10 g/kg of toxic material five times a week, Pig F1 showed a greater tolerance. Anorexia was first noticed in Pig F2 from the 3rd day and was complete by Day 5. From the 4th day scleral icterus was apparent. By the afternoon of the 6th day it was found to be very weak and staggered around in its pen, being partially inco-ordinated. Respiration was laboured and it made weak moaning sounds. It died that night. Its weight dropped from 39 to 38 kg during this time. Pig F1 was still feeding a little by the 8th day and was reasonably lively. By the 18th day it was very icteric, listless, anorectic and showed polydipsia. Weight loss amounted to 3 kg from Day 1 to 12 when it was killed *in extremis*.

Pigs F3 and F4 weighed 40 and 41 kg respectively at the commencement of toxin administration. One week later both showed severe loss of weight and weighed 34 and 37 kg respectively. Anorexia was more pronounced in Pig F4 than Pig F3 at this stage, and by the 10th day it remained prone most of the time and was inappetent. Its voice was much weaker than normal. By the 11th day a very slight icterus was detectable in the sclera. It became gradually weaker, drank but did not eat and died during the night of the 13th day when it weighed 30 kg, *i.e.*, a loss of 11 kg over a 13 day period.

Pig F3 showed severe polydipsia from the 8th day while anorexia increased. On the 14th day it weighed 28 kg, *i.e.* a loss of 11 kg over the 2 week period which was identical to that of Pig F4. By the 16th day anorexia was complete, it only drank water and was jaundiced. On the 17th day it was *in extremis*, very icteric, and walked with swaying, partially incoordinated hindquarters. The ears and snout were intensely congested and cold to touch. It was killed for necropsy at this stage.

Pigs F5 and F6 weighed 36 and 41 kg respectively at the commencement of toxin administration. Pig F5 became less interested in feeding from the 5th day. On the 8th day it weighed 28 kg and appeared very weak and gaunt but no sign of icterus was present. It lingered on like this eating almost nothing and drinking very little water. The faeces became dry and hard. After 2 weeks it weighed only 23½ kg and by the 5th week, 18 kg. It eventually became dehydrated, was unable to get up unaided and showed weakness especially of the hindquarters and finally died after 6 weeks weighing only 16 kg. Pig F6 responded severely to the toxin and became anorectic on Day 5. It had lost 8 kg of weight during the first week and was very weak but not icteric. On Day 8 anorexia was complete, it had poor control over its hindlimbs and assumed a dog-sitting position. Toxin administration was suspended for 1 week and on Day 14 it appeared clinically normal and regained much of its weight, *i.e.*, it weighed 35 kg. At this stage toxin was again administered. The pig seemed in good health the following week and in fact gained more weight until it weighed almost 41 kg 4 weeks after the first administration. From then toxin was administered at weekly intervals. In the 6th week anorexia again set in. The hindquarters swayed when walking but the habitus remained good. Body weight dropped by 2 kg. Its condition deteriorated rapidly in the 7th week, the hindquarters became very inco-ordinated and eventually almost completely paralysed. The skin was very hyperaemic and tachycardia was present (its pulse rate was 164 per minute). At this stage it was killed for necropsy.

The control Pigs F7 and F8 did very well and gained weight from 41 to 47 kg and 41 to 45 kg respectively in the 1st week. Unfortunately Pig F7 suffered a blowfly strike in the surgical wound during the second week which led to peritonitis and it had to be discharged from the experiment. Pig 8 weighed 80 kg when it was slaughtered during the 7th week of the experiment. The autoclaved maize kernels did not have any

detrimental effect as far as could be ascertained. Details of variations in body weight of some of the pigs in Experiment II are given in Fig. 12.

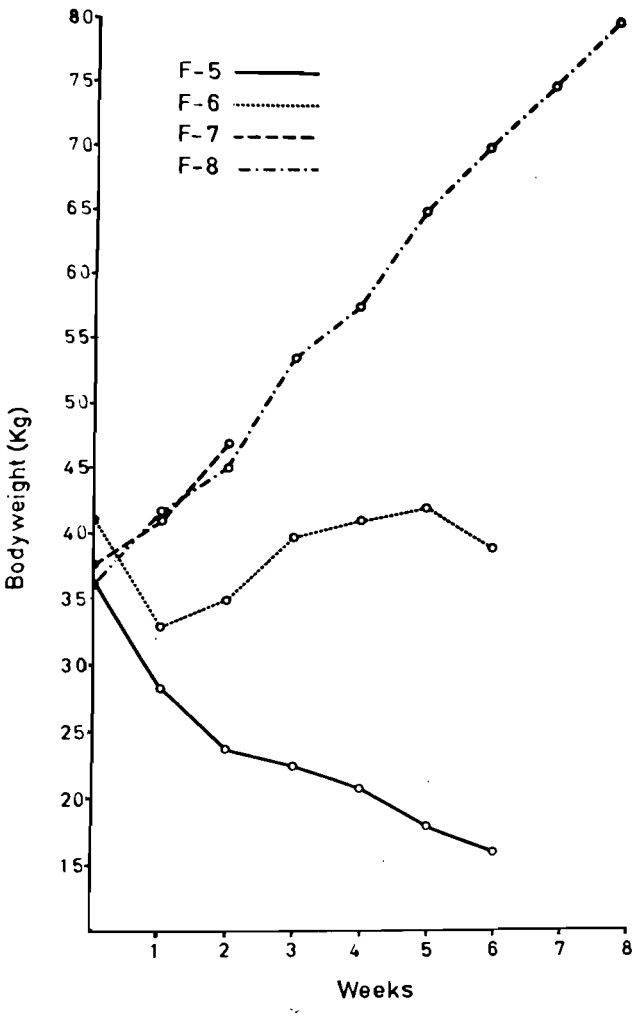


Fig. 12: Graphic presentation of changes in body weight encountered in pigs surviving more than 2 weeks in Experiment II.

GROSS PATHOLOGY

Pig F1 showed severe generalised icterus and had an orange-brown, atrophied liver which was increased in consistency. The bile was very viscous. There was a nephrosis, the kidneys being swollen and a very light brown colour. The urine was brownish yellow and contained a considerable quantity of yellow floccular precipitate. The small and large intestinal mucosa was diffusely hyperaemic. The carcass of Pig F2 was cyanotic, congested and icteric. The liver was not atrophic and the bile was yellow-green and more gel than fluid in nature with floccules in it. Its volume was not increased. The intestinal contents contained a small amount of free blood mixed with the contents. A moderate hydropericardium (± 25 ml) was present.

Of Pigs F3 and F4 the former lived ionger and showed a more severe terminal icterus which was especially conspicuous in the intima of the blood vessels and in the sclera and fascia layers which were ochre in colour. The skin was yellow, but the subcutaneous fat was more a dirty pink due to congestion and revealed little of the icterus. The liver was also ochre in colour, and was slightly atrophic with a slight increase in consistency as opposed to a normal-sized brown liver with distinct lobulation in Pig F4. The bile in both pigs was a very thick slimy material. Both pigs were constipated and had extensive suggilations in the intestinal wall while severe enterorrhagia was present only in F4. In both there was a mild nephrosis but the urine was normal in appearance.

The lesions in Pigs F5 and F6 differed considerably. Pig F5 was dehydrated, emaciated, icteric and showed haemoconcentration. Although the liver was slightly atrophic its consistency was not firmer than normal. Its colour was orange-brown. The bile resembled that of Pigs F2 and F4. Numerous focal ecchymoses occurred in the intestinal wall with much free blood in the lumen of the gut. The urine was brown (but no floccules were present in it.) Subendocardial ecchymoses were present. The carcass of Pig 6 was slightly icteric and had a bled-out anaemic appearance. There was a severe haemoperitoneum with many haemorrhages in the omentum and mesentery and subperitoneally on the diaphragm. Numerous haemorrhages of 0,5 — 1cm in diameter were disseminated throughout both lungs. No free blood was present in the intestine. The blood in the heart and elsewhere was unclotted at the time of necropsy despite an interim between death and autopsy of approximately 10 hours. The liver was a light orange-pink and the consistency was not increased. The bile was a yellow-orange, floccular viscous material. The urine contained a floccular sediment.

Pig 8 was normal in all respects except for the presence of the rubber fistula in the stomach.

HISTOPATHOLOGY

Generally speaking the histopathological changes encountered in the fistulated pigs were similar to those seen in Experiment I. It seemed therefore that the effect did not differ significantly whether toxin was administered as a single dose or repeatedly. In Pig F1 the larger bile ducts were filled with a pink proteinaceous mucoid like material. The kidney changes in Pigs F1 and F2 were of a similar nature but were much more severe than those in any other pig and furthermore were more advanced than the hepatic lesions in F2. The nuclei of some convoluted tubular epithelial cells contained pseudo-inclusions similar to those mentioned above in the liver while the cytoplasm was condensed into an eosinophilic globule in others. Some nuclei were enlarged. A few cells were binucleate. The changes therefore were similar to those observed in hepatocytes. The descending and ascending loops of Henle contained protein rich pink casts. The epithelium of the collecting tubules was hypertrophied and proliferative in areas — several cells having more than one nucleus. In some the nuclei were pyknotic while those of others were in mitosis. Several tubular epithelial cells had a foamy cytoplasm proximal to the nucleus. Necrotic cellular debris occurred in the lumen of several ducts.

In the myocardium of F1 were numerous focal areas of degeneration in which muscle fibres were more eosinophilic than normal and cross striations indistinct.

The lesions in Pigs F3 and F4 followed a similar pattern to the abovementioned in that the kidney of F4 was much more severely affected than the liver. In the liver of Pig F3 parenchymal cells containing

eosinophilic globules were more numerous than in any of the other pigs. The nuclei of several hepatocytes consisted merely of a rim of chromatin surrounding a clear space and contained a marginally situated nucleolus. The perinuclear cytoplasm in most cells was intensely eosinophilic and granular while at the periphery it had a more basophilic and foamy appearance. The major changes in the liver of Pig F4 included bi- and multinucleism, variation in nuclear size, and the presence of intranuclear pseudo-inclusions. Most nuclei exhibited irregularly folded membranes. The kidneys were more severely affected than the liver and showed degenerative and necrotic changes of the tubular epithelium with desquamation and the formation of cellular casts in the tubules resembling those of Pigs F1 and F2. Some desquamated epithelial cells, however, were not necrotic and occurred in clusters in the widened lumens of affected tubules. Mitotic figures were present in the cortex and medulla and it appeared as if most of the desquamated cells were replaced by flatter epithelial cells in a process of regeneration. Some cells contained single large or several smaller nuclei. Eosinophilic droplets occurred in the lumens of many tubules.

The myocardium of Pig F3 had focal areas of rarifaction and of more eosinophilic staining shrunken fibres.

In Pigs F5 and F6 the former showed lesions more of the acute type. In the liver the nuclear abberations were very conspicuous, some nuclei being large and irregularly shaped. The majority, however, had a crenated appearance and several contained large pseudo-inclusions. In the kidneys bilirubin casts were present in the lumens of several tubules and focal areas of slight fibrosis associated with a mild round cell infiltration occurred in the cortex. In Pig F6 the liver was severely affected and the lesions more or less resembled those of the pig examined in the pilot trial. Most hepatocytes had disappeared and only a few islands of these cells were left in the lobules. They were surrounded by loose connective tissue. These hepatocytes tended to assume an acinar arrangement (Figs. 5&6). Some contained fat droplets or yellow-brown pigment globules. Several were binucleate while others had large vesicular or large irregular shaped nuclei. Unfortunately *post mortem* changes were fairly advanced and obscured the renal pathology.

The mucosa of the gall bladder was focally infiltrated by round cells in Pigs F1 and F3. There was a severe congestion with focal microscopic haemorrhages in the brain and spinal cord of Pigs F2, F4, F5 and F6. Large focal haemorrhages occurred in the lungs of Pig F6.

DISCUSSION

These experiments have conclusively demonstrated that pigs are susceptible to intoxication by the mycotoxin produced by *P. leptostromiformis* although due to the anorexia produced by the toxin it is improbable that many acute deaths will occur under natural circumstances following ingestion of sublethal amounts. The growth stunting effect of the toxin is significant, however, and its presence in pig rations could lead to serious economical losses. It is therefore essential that lupins used for pig feed should be uncontaminated.

The results obtained in these experiments indicate that the mycotoxin affects primarily the liver, kidneys and myocardium of pigs. In animals receiving relatively large amounts of toxic material the hepatic changes were primarily degenerative and necrotic in nature together with severe nuclear abnormalities. An interesting observation was the rapidity of the development of hepatic fibroplasia following mycotoxin ingestion. In Pig F1 there was a definite fibrosis present within 7 days. It is also interesting to note that in pigs the toxin did not induce nearly as severe a degree of fatty change or bile duct proliferation in the livers as it does in sheep[1 4 5 6 18 19] cattle[1 2] and mice[3 4 7 10 13 14]. Liver reactions similar to those in other species, however, were the presence of pseudo-inclusions in the nuclei, binucleate cells, large bizarre nuclei and eosinophilic globules and pigment in the cytoplasm of hepatocytes. Those pigs exposed to smaller doses exhibited lesions of a more chronic nature manifested by the replacement of hepatocytes with anaplastic cells which tended to be arranged in acini. The renal changes were mainly necrosis of epithelium particularly of the proximal and distal convoluted tubules. The renal damage produced by the toxin is more severe in pigs than in any of the other animal species mentioned.

Only one reference has been encountered concerning the myocardial damage caused by the mycotoxin of *P. leptostromiformis* in sheep[19]. It has, however, been the experience of one of us (I.B.J.V.R.) that in the majority of experimental cases of intoxication with this mycotoxin in sheep, rabbits, guinea pigs and mice, severe myocardial lesions have been present. In this series of the disease in pigs six of the 11 animals used showed focal areas of myocardial hyalin degeneration and/or necrosis.

The liver lesions, particularly those of the animal in the pilot experiment in which the primitive appearance and acinar arrangement of hepatocytes as well as the nuclear changes of these cells were prominent, compel one to consider the possibility of this mycotoxin being carcinogenic. Pigs have recently been proved susceptible to the hepatocarcinogenic activity of aflatoxin [16].

No explanation can be offered for the finding that the weight loss induced by the toxin is not positively correlated to the dosage. Pigs F3 and F4 which received the "medium" dosage showed by far the most severe and rapid loss of weight.

No lesions were detected in the central nervous systems which could have accounted for the posterior paresis which was a constant and rather severe clinical sign.

The preliminary results obtained in these experiments do not indicate any difference between gilts and castrated males in susceptibility to the mycotoxin.

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