RESULTS

Production of the crude toxins and PES

Ability of various strains of A. *niger* of producing the crude toxins is summarized in Table 1. It was found that these strains of A. *niger* produced a wide range of the amount of the crude toxins from 0.15-2.58 g/kg moldy rice. It can be divided according to the productive capability into 3 groups with high capability (1.50-2.58 g/kg moldy rice) moderate capability (1.00-1.49 g/kg moldy rice) and low capability (0.15-0.99 g/kg moldy rice). Most of the fungal isolates (46.2%) which were in the third group produced the crude toxins in the low amount of approximate 0.61 g/kg moldy rice. However, the average production of the crude toxins from all fungal isolates of A. *niger* was approximate 1.09 g/kg moldy rice. Table 2 shows the ability of fungal isolates from other species of producing the crude toxins. Similar results were obtained with the productive capability of the crude toxins of 1.23 g/kg moldy rice and the fungal isolates of A. *fumigatus* produced the higher yield of the crude toxins of approximate 1.66 g/kg moldy rice. Furthermore, it was noted that chloroform and methylene chloride could be equally used to extract the moldy rice without any significantly differences in the given yield.

A similar finding on the ability of various strains of A. *niger* of producing the PES was also observed. It is summarized in Table 3. It was found that these strains of A. *niger* produced

TABLE 1

Production of the crude toxins by various strains of A. *niger* on glutinous rice for 14 to 21 days at 25±1°C.

Fungal isolate[a]	Extraction of moldy rice		Crude Toxin (g/kg moldy rice)
	Solvent	Number[b]	
AN-004-, -031-, -034-, 073-, -A30-, -A31-75	$CHCL_3$	5	1.50, 1.80, 1.82
	$CHCL_2$	1	1.76, 2.58, 1.66
			Mean = 1.85
			Range = 1.50-2.58
AN-008, -012, -030-, -047-, -064-, -072-, -082-, -121-75	$CHCL_3$	4	1.20, 1.08, 1.26
	$CHCL_2$	4	1.24, 1.16, 1.42, 1.20, 1.26
			Mean = 1.23
			Range = 1.08-1.49
AN-010-, -014-, -020-, -022-, -025-, -051-, -058-, 067-, -068-, -A17-, -A26-75	$CHCL_3$	11	0.19, 0.15, 0.80, 0.50, 0.80, 0.82 0.98, 0.50, 0.75 0.80, 0.40, 0.60,
	$CHCL_2$	1	
			Mean = 0.61
			Range = 0.15-0.98

Total fungal isolates = 26

Mean of the crude toxins = 1.09 g/kg moldy rice.

000107

[a] AN-000-75 = Culture number of the fungal isolates

[b] Number of the fungal isolate extracted by $CHCL_3$ or $CHCL_2$.

TABLE 2

Production of the crude toxins by the fungal isolates from other species on glutinous rice for 14 to 21 days at 25±1°C.

Fungal isolate[a]	Extraction of moldy rice		Crude Toxin (g/kg moldy rice)
	Solvent	Number[b]	
AC-007-, -019-, -023-75	$CHCL_3$	3	1.38, 1.78, 0.24 Mean = 1.13 Range = 0.24-1.78
ACLA-055-75	$CHCL_3$	1	0.98
AFU-007-, -025-, -030-, -119-75	$CHCL_3$	4	1.40, 1.62, 1.00, 2.62 Mean = 1.66 Range = 1.00-2.62
AND-005-75	$CHCL_3$	1	0.20
P(g)-048-, (py)-061-75	$CHCL_3$	2	1.38, 0.92 Mean = 1.15 Range = 0.92-1.38

Total fungal isolates = 11

Mean of the crude toxins = 1.23 g/kg moldy rice

[a] AC, ACLA, AFU, and P-000-75 = Culture number of the fungal isolates (AC = A. *candidus*, ACLA = A. *clavatus*, AFU = A. *fumigatus*, AND = A. *nidulans* and P = *Penicillium*)

[b] Number of the fungal isolate extracted by $CHCL_3$ or $CHCL_2$.

TABLE 3

Production of the PES by various strains of A. *niger* on glutinous rice for 14 to 21 days at 25±1°C.

Fungal isolate[a]	Extraction of moldy rice		PES
	Solvent	Number[b]	(ml/kg moldy rice)
AN-A31-, -051-75	$CHCL_3$	2	11.40, 11.0
			Mean = 11.20
			Range = 11.0-11.40
AN-010-, -020-, -021-,	$CHCL_3$	11	5.40, 6.00, 9.00,
-030-, -034-, -052-,	$CHCL_2$	1	6.60, 7.40, 7.00
-058-, -067-, -068-,			6.80, 7.80, 5.80,
-082-, -A17-, -A30-75			7.20, 9.60, 8.00
			Mean = 7.22
			Range = 5.40-9.60
AN-004-, -008-, -009-,	$CHCL_3$	7	2.60, 2.60, 4.40
-012-, -014-, -022-,	$CHCL_2$	5	4.80, 3.80, 3.00
-025-, -031-, -038-,			2.60, 4.60, 1.60
-047-, -064-, -072-,			3.20, 3.40, 4.40
-073-, -A26-, -121-75			2.20, 4.60, 2.00
			Mean = 3.32
			Range = 1.60-4.80

Total fungal isolates = 26

Mean of the PES = 6.11 ml/kg moldy rice

[a] AN-000-75 = Culture number of the fungal isolates

[b] Number of the fungal isolate extracted by $CHCL_3$ or $CHCL_2$.

a wide range of the amount of the PES from 1.60-11.40 ml/kg moldy rice. The average production of the PES from all fungal isolates of A. *niger* was approximate 6.11 ml/kg moldy rice. The average production of the PES by the fungal isolates from other species was found to be higher of 10.4 ml/kg moldy rice (Table 4).

Content of aflatoxins in the crude toxins

The content of aflatoxins in the crude toxins produced by the fungal isolates of A. *niger* and other species is summarized in Table 5. A total of 6 out of 30 fungal isolates of A. *niger* produced only small amount of aflatoxins B_1 and B_2 with the average amount of 16 ppb and trace respectively. The higher content of aflatoxins B_1 and B_2 was found in the crude toxins produced by A. *fumigatus* (3/4) with the average amount of 462 ppb and 6 ppb respectively. However, no aflatoxins G_1 and G_2 were detected in the crude toxins produced by these strains. No detectable amount of aflatoxins was observed in the crude toxins produced by other strains except A. *niger* and A. *fumigatus*.

Content of oxalate in the crude toxins

The content of oxalate in the crude toxins produced by the fungal isolates from A. *niger* and other species in summarized in Table 6. It was found that the rather small amount of oxalate was presented in the crude toxins produced by various strains of A. *niger* and other species. Higher oxalate content were found in the crude toxin produced by fungal isolates of AN-022-75 and AN-121-75 of 170 and 144 µg/100 mg crude toxin respectively. Mean oxalate content in all crude toxins analysed was approximate 39.9 µg/100 mg crude toxin.

TABLE 4

Production of the PES by the fungal isolates from other species on glutinous rice for 14 to 21 days at 25±1°C.

Fungal isolate[a]	Extraction of moldy rice		PES (ml/kg moldy rice)
	Solvent	Number[b]	
AC-007-, -019-, -023-75	$CHCL_3$	3	5.80, 5.00, 2.60 Mean = 4.47 Range = 2.60-5.80
ACLA-055-75	$CHCL_3$	1	18.80
AFU-007-, -025-, -030-, -119-75	$CHCL_3$	4	6.20, 5.80, 6.80 6.00 Mean = 6.20 Range = 5.80-6.80
AND-005-75	$CHCL_3$	1	25.00
P(g)-048-, (py)-061-75	$CHCL_3$	2	14.20, 18.20 Mean = 16.20 Range = 14.20-18.20

Total fungal isolate = 11

Mean of the PES = 10.4 ml/kg moldy rice

[a] AC, ACLA, AFU, AND and P-000-75 = Culture numbers of the fungal isolates (AC = A. *candidus*, ACLA = A. *clavatus*, AFU = A. *fumigatus*, AND = A. *nidulans* and P = *Penicillium*)

[b] Number of the fungal isolate extracted by $CHCL_3$ or $CHCL_2$.

TABLE 5

Content of aflatoxins in the crude toxins produced by various strains of A. *niger* and other species.

Species	Number of Producing Strains/examined	Mean aflatoxin (ppb)[a]			
		B_1	B_2	G_1	G_2
A. *niger* (AN)	6/30	16	trace	ND	ND
A. *fumigatus* (AFU)	3/4	462	6	ND	ND
A. *candidus* (AC)	0/2	ND	ND	ND	ND
A. *clavatus* (ACLA)	0/1	ND	ND	ND	ND
A. *nidulans* (AND)	0/1	ND	ND	ND	ND
Penicillium (P)	0/2	ND	ND	ND	ND

[a] Ranges of aflatoxin production : B_1, trace - 34 ppb; B_2, ND-trace from A. *niger* and B_1, 20-1300 ppb; B_2, ND-14 ppb from A. *fumigatus*.

ND = non-detected

tract = aflatoxin <1 ppb

ppb = parts per billion (µg/kg moldy rice)

TABLE 6

Content of oxalate in the crude toxins produced by various strains of A. *niger* and other species.

Fungal isolate[a]	Oxalate (µg/100 mg crude toxin)[b]
AN-022-, -121-75	170, 144
	Mean = 157
	Range = 144-170
AN-008-, -009-, -030-, -034-,	14, 15, 44, 24,
-038-, -047-, -051-, -052-,	54, 30, 10, 80
-058-, -064-, -067-, -073-,	44, 33, 20, 15,
-A26-, -A31-75	20, 10
	Mean = 29.5
	Range = 10-80
P(g)-061-75	20
AND-005-75	10
AFU-007-75	10
Other species	ND

Total fungal isolates of A. *niger* = 26

Total fungal isolates of other species = 11

Mean of the oxalate = 39.9 µg/100 mg crude toxin

[a] AN, P, AND and AFU-000-75 = Culture numbers of the fungal isolates (AN = A. *niger* P = *Penicillium*, AND = A. *nidulans* and AFU = A. *fumigatus*).

[b] ND = non detectable

Acute toxicity tests of the crude toxins and PES

Results of the bioassay of the crude toxins produced by various strains of A. *niger* in the weanling female rats revealed that nearly 69.3% of these isolates were toxic to the rats (Table 7). In the standard toxicity test using weanling rats in the bioassay of the crude toxins of the dose equivalent to 50 g of moldy rice is used. In this study, it was found that most of the toxic crude toxins (58.8%) were very potent at the dose equivalent to 12.5 g of moldy rice. These isolates were AN-030-75, AN-051-75, AN-052-75, AN-067-75, AN-022-75, AN-A26-75 and AN-A31-75. AN-121-75 and AN-A30-75 isolates were considered to be the moderately toxic. The tested animals that survive from the treatment of the lethal dose had a considered slow body weight gain. In addition, some of the non-toxic crude toxin also caused a reduction in the growth rate of the tested animals, even though the rats were survived throughout the course of experiment. The higher toxicity of the crude toxins produced by other species was observed (Table 8). Approximate 90.0% of all fungal isolated from other species was highly toxic to the rats. The most toxic isolate was AC-019-75 which the lethal dose of the crude toxin was found to be equivalent to 6.25 g of moldy rice. The least toxic isolates was AC-023-75 with the lethal dose equivalent to 25.0 g of moldy rice. However, AFU-007-75 isolate caused a body weight loss in the tested animal.

Results of the bioassay of the PES produced by various strains of A. *niger* in the weanling female rats revealed that only 22.0% of these isolates were toxic to the rats (Table 9).

TABLE 7

Acute toxicity of the crude toxins produced by various strains of A. *niger* on weanling female rats given ip.

Fungal isolate[a]	Mortality (dead/tested)				Body weight[c] change (g)
	Dose equivalent to g moldy rice				
	50.0	25.0	12.5	6.25	
AN-008-[b], -012-, -020-, -064-, -082-75	5/5	5/5	-	-	-
AN-030-, -051-, -052-, -067-75	-	5/5	5/5	-	-
AN-022[b]-, -A26-, -A31-75	3/3	3/3	3/3	-	-
AN-034-75	3/3	2/3	2/3	-	+ 15.5
AN-058-, -068-75	3/3	3/3	1/3	-	+ 22.7, + 26.1
AN-121-75	-	4/5	-	-	-
AN-A30-75	3/3	3/3	0/3	-	+ 13.7
AN-004-75	4/5	0/5	0/5	-	
AN-047-75	0/5	0/5	-	-	+ 11.8, + 13.9
AN-A17-, -072-75	0/3	0/3	0/3	-	+ 33.9, + 25.2
AN-010-, -014-, -025-75	0/5	0/5	-	-	-
AN-031-, -073-75	0/5	0/4	0/4	-	+ 24.0, + 18.8

Toxic crude toxins = 18/26 (69.23%)

Toxic crude toxins at dose level of 12.5 g moldy rice = 10/26 (38.46%)

[a] AN-000-75 = Culture number of the fungal isolates

[b] Aflatoxin - producing strain

[c] Body weight change (g) : + = gain and - = loss.

TABLE 8

Acute toxicity of the crude toxins produced by various strains of other species on weanling female rats given ip.

Fungal isolate[a]	Mortality (dead/tested)				Body weight[c] change (g)
	Dose equivalent to g moldy rice				
	50.0	25.0	12.5	6.25	
AC-019-75	-	5/5	5/5	5/5	-
AFU-007-75	5/5	5/5	5/5	4/5	-9.82
AFU-119-75[b]	5/5	5/5	5/5	2/5	+20.0
ACLA-055-75	5/5	5/5	4/5	2/5	+25.6
AC-007-75	5/5	5/5	5/5	0/5	+20.9
AFU-025-75[b]	5/5	5/5	5/5	-	-
P (g)-048-75	5/5	5/5	5/5	1/5	+21.0
AFU-030-75[b]	5/5	5/5	3/5	-	+15.7
P (py)-061-75	5/5	5/5	4/5	2/5	+25.6
AC-023-75	-	5/5	0/5	-	-
AND-005-75	-	-	-	-	-

Toxic crude toxins = 10/11 (90.91%)

Toxic crude toxins at dose level of 6.25 g moldy rice 6/11 (54.55%)

[a] AC, ACLA, AFU, AND and P-000-75 = Culture number of the fungal isolates (AC = A. *candidus*, ACLA = A. *clavatus*, AFU = A. *fumigatus*, AND = A. *nidulans* and P = *Penicillium*).

AND crude toxin was not tested because the small amount of the crude toxin.

[b] Aflatoxin - producing strains.

[c] Body weight change (g) : + = gain and - = loss.

TABLE 9

Acute toxicity of the PES produced by various strains of *A. niger* on weanling female rats given po.

Fungal isolate[a]	Dose of PES (ml/rat)	Mortality (dead/tested)	Body weight[c] change (g)
AN-004-75	0.33	3/3	-
AN-064-75	0.80	3/3	-
AN-010-75	0.90	3/3	-
AN-021-75	0.90	3/3	-
AN-030-75	1.00	3/3	-
AN-051-75	1.00	3/3	-
AN-034-75	1.00	1/3	+20.5
AN-073-, -038-, -121-,	0.20, 0.25, 0.33	0/3	+22.4, +18.4, -,
-022[b]-, -025-, -031-,	0.40, 0.40, 0.40		+30.8, -, +24.0
-008[b]-75	0.43		+7.7
AN-047-, -074-, -072-	0.53, 0.60, 0.60	0/3	+26.0, +15.0,+40.8
-A26-, -009-, -012-	0.60, 0.70, 0.80		+33.6, -, +24.9
-058-75	0.80		+32.2
AN-082-, -A17-,	1.00, 1.00, 1.00	0/3	+6.8, +34.0
-A30-, -A31-, -052-,	1.00, 1.00, 1.00		+14.2, +24.1,-.
-067-75			

Toxic PES = 7/27 (25.93%)

[a] AN-000-75 = Culture number of the fungal isolates

[b] Aflatoxin - producing strains

[c] Body weight change (g) : + = gain and - = loss.

These isolates were AN-004-75, AN-064-75, AN-010-75, AN-021-75, AN-030-75 and AN-051-75. The most toxic isolate was AN-004-75 with the lethal dose of the PES at 0.33 ml/rat. In addition, nearly 64.0% of all isolates from other species produced toxic PES (Table 10). The potency of the PES from these isolates was similar to the PEI from various strains of A. *niger*.

General observations on treated animals.

After administration of the crude toxins and PES, the survival time and the clinical signs were recorded. The period of the survival time in the animals treated with various doses of the crude toxins and PES can be divided into three groups of a few hours (1-4 hours) in the first group, a few hours after the high dose and a few days (1-3 days) after the lower dose in the second group and a few days after all doses in the third group. It was found that there was only 4 strains of the fungal isolates, AN-051-75 (PES), AC-007-75, ACLA-055-75 and P (py)-061-75 produced the lethal crude toxins and PES in the animal that survive in the period of first group. Approximate 51.9% and 37.0% of the crude toxins produced by various strains of A. *niger* and other strains caused death to the animals in the period of second and third groups respectively. However, most of the PES (90.7%) produced by various strains of A. *niger* (AN-009-75, AN-010-75, AN-021-75, AN-030-75, AN-051-75 and AN-068-75) and other strains (AC-007-75, AC-023-75, ACLA-055-75, AFU-119-75, P (g)-048-75 and P (py)-061-75 caused death to the animals in the period of third group.

Animals given high doses of the crude toxins or PES died within 1-3 hours. Most of these animals became ataxic, drowsy

TABLE 10

Acute toxicity of the PES produced by various strains of other species on weanling female rats given po.

Fungal isolate[a]	Dose of PES (ml/rat)	Mortality (dead/tested)	Body weight[c] change (g)
AC-023-75	0.40	3/3	-
AC-007-75	0.80	3/3	-
ACLA-055-75	1.00	3/3	-
P(g)-048-75	1.00	3/3	-
P(py)-061-75	1.00	3/3	-
AFU-007-75	1.00	1/3	+15.6
AFU-119-75[b]	1.00	1/3	+33.2
AC-019-75	1.00	0/3	+ 6.5
AFU-025-75[b]	1.00	0/3	+23.9
AFU-030-75[b]	1.00	0/3	+26.9
AND-005-75	1.00	0/3	+ 9.5

[a] AC, ACLA, AFU, AND and P-000-75 = Culture number of the fungal isolates (AC = A. *candidus*, ACLA = A. *clavatus*, AFU = A. *fumigatus*, AND = A. *nidulans* and P = *Penicillium*)

[b] Aflatoxin - producing strains

[c] Body weight change (g) : + = gain and - = loss.

and finally lied down on the bottom of the cage and became sudden comatose. There was only a few of the crude toxins produced by AFU-007-75, AFU-025-75 and AFU-030-75 that could cause convulsions. Those animals that died within a few days usually consumed less amount of food and then died.

Microscopic examination

The results of the histopathologic examinations of tissues taken from the rats that died in the toxicity tests of the crude toxins and PES are given below in two parts. First part deals with the number of the crude toxins and PES that can cause histopathologic changes in liver, kidney, lung, pancreas and other organs. These changes are: liver, necrosis, fatty degeneration, acidophilic cytoplasm and congestion; kidney, necrosis in the tubular cells, dilatation of the tubules and congestion; lung, congestion and mild pneumonitis; pancreas, necrosis and spleen, mostly congestion. Second part deals with the representative histopathologic changes in various organs of the rats treated with the crude toxins and PES produced by various strains of A. *niger* and other species.

Table 11 presents the summary of the possible target organs of weanling female rats treated with the lethal dose of the crude toxins from A. *niger*. It was found that only 23.5% of the crude toxins produced histopathologic changes of mostly mild pycnotic and acidophilic cytoplasm in the liver. However, most of the crude toxins (94.1%) produced histopathologic changes of mostly various degrees of necrosis in tubular cells of kidney,

whereas mostly congestion was found in the lung and spleen. Necrosis of the pancreatic cells was found in two crude toxins produced by AN-008-75 and AN-121-75.

Summary of the possible target organs of weanling female rats treated with the lethal dose of the crude toxins and PES from A. *niger* and other strains is shown in Table 12. Similar results were obtained with most of the crude toxins caused renal damages and no toxic effect was observed in pancreas. The severe necrosis of liver and kidney was observed in the rats treated with PES from AN-010-75 and AC-023-75.

Representative histopathologic changes are presented below:

AN-004-75: Histopathologic examinations of various organs of weanling female rats died on the first day after ip administration of PEI (25.0 g moldy rice) produced by A. *niger* (AN-004-75).

Liver: Histopathologic changes revealed moderate fatty degeneration of the hepatic cells in the midzonal areas of liver in one animal and other showed an irregularity of the nuclear sizes.

Kidney: congestion was mostly observed in the cortex and corticomedullary zones of kidney. Necrosis of the tubular cells of various convoluted tubules was also observed in these zones and sometimes it extended into the medullary zones. Epithelial cells were gone to the necrotic processes and sloughly off into the lumen of the convoluted tubules (Fig. 1A and B). In addition hyaline casts in the lumen of these convoluted tubules were also observed.

TABLE 11

Summary of the possible target organs of weanling female rats treated with the lethal dose of the crude toxins from *A. niger*.

Fungal isolates[a]	Possible target organ (positive/examined)[c]				
	Liver	Kidney	Lung	Pancreas	Others
AN-008-75[b]	0/3	3/3	3/3	1/3	Spleen 2/3
AN-012-75	0/3	3/3	2/3	0/3	-
AN-020-75	1/3	3/3	1/3	0/3	Spleen 1/3
AN-064-75	1/3	3/3	1/3	0/3	Spleen 2/3
AN-082-75	1/3	2/3	0/3	0/3	-
AN-030-75	0/3	3/3	3/3	0/3	-
AN-051-75	0/3	2/3	0/3	0/3	Spleen 2/3
AN-052-75	0/3	3/3	0/3	0/3	-
AN-067-75	0/3	3/3	0/3	0/3	Spleen 2/3
AN-022-75	0/3	1/3	1/3	0/3	-
AN-A26-75	0/3	1/3	0/3	0/3	-
AN-A31-75	0/3	2/3	0/3	0/3	-
AN-034-75	0/3	0/3	1/3	0/3	-
AN-058-75	0/3	2/3	0/3	0/3	Spleen 1/3
AN-068-75	3/3	1/3	2/3	0/3	-
AN-121-75	0/3	1/3	2/3	1/3	-
AN-A30-75	0/3	2/3	1/3	0/3	-
Total toxic crude toxins cause organ damages	4/17 (23.5%)	16/17 (94.1%)	10/17 (58.8%)	2/17 (11.8%)	Spleen 6/17 (35.3%)

[a] AN-000-75 = Culture number of the fungal isolates

[b] Aflatoxin - producing strain

[c] Histopathologic changes (see text) in various organs/examined.

TABLE 12

Summary of the possible target organs of weanling female rat treated with the lethal dose of the crude toxins and PES from *A. niger* and other strains.

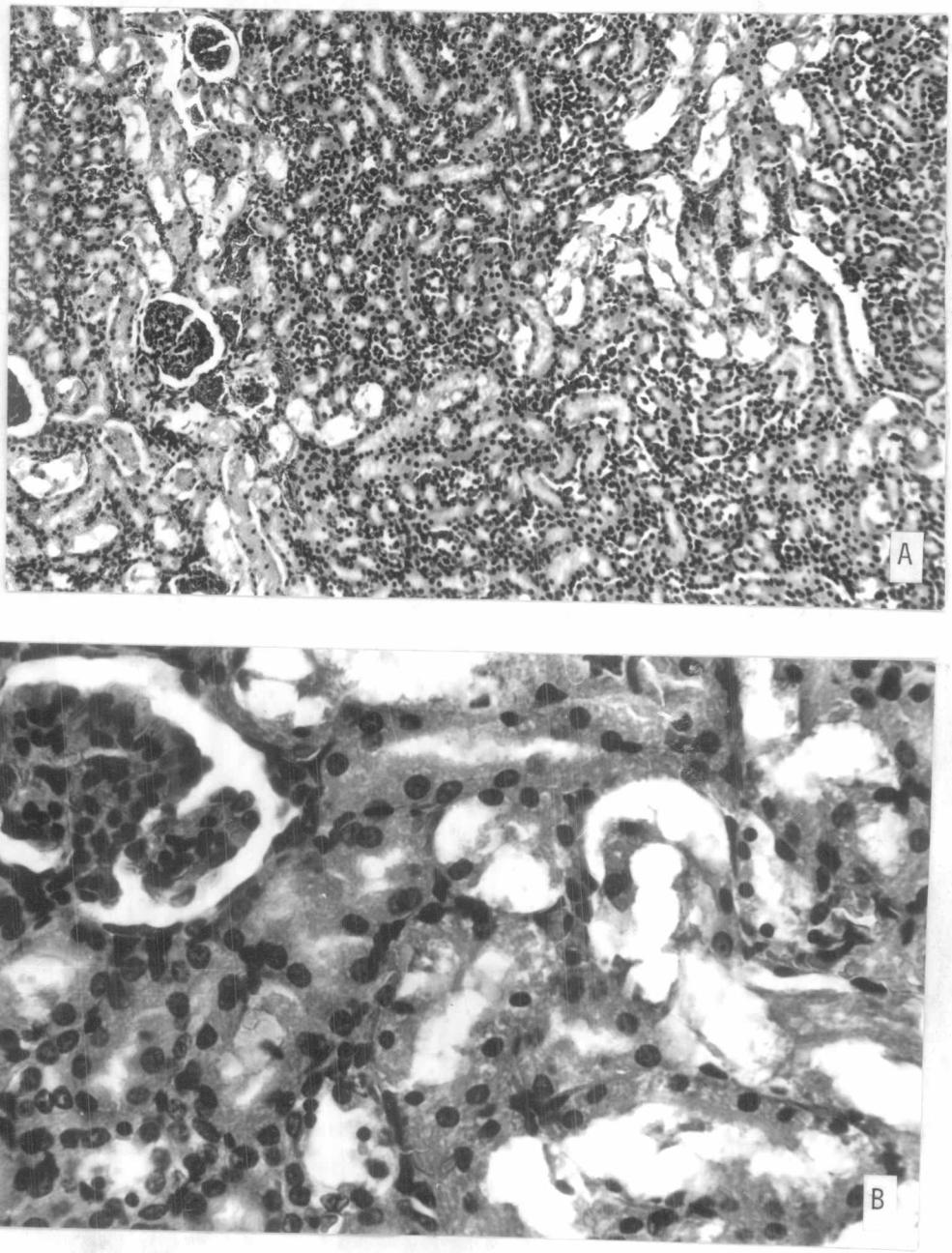
Fungal isolates[a]	Possible target organ (positive/examined)[c]				
	Liver	Kidney	Lung	Pancreas	Others
Crude toxin					
AC-019-75	1/3	0/3	0/3	0/3	-
AC-023-75	0/3	3/3	0/3	0/3	-
AC-007-75	0/3	1/3	0/3	0/3	-
ACLA-055-75	0/3	2/3	2/3	0/3	Spleen 2/3
AFU-007-75	0/3	2/3	2/3	0/3	-
AFU-030-75[b]	1/3	1/3	1/3	0/3	-
AFU-119-75[b]	0/3	2/3	1/3	0/3	Spleen 2/3
AFU-025-75[b]	0/3	2/3	0/3	0/3	Spleen 3/3
P(g)-048-75	0/3	3/3	2/3	0/3	-
P(py)-061-75	1/3	2/3	2/3	0/3	-
Total toxic crude toxins cause organ damages	3/10 (30.0%)	9/10 (90.0%)	6/10 (60.0%)	0/10 (00.0%)	3/10 (30.0%)
PES					
AN-009-75	0/3	0/3	0/3	0/3	-
AN-010-75	2/3	2/3	1/3	0/3	-
AN-030-75	0/3	0/3	0/3	0/3	-
AN-034-75	0/3	1/3	0/3	0/3	-
AC-023-75	2/3	3/3	2/3	0/3	-

[a] AN-000-75 = Culture number of the fungal isolates

[b] Aflatoxin - producing strain.

Figure 1

Kidney of weanling female rat died on the second day after ip administration of PEI (25.0 g moldy rice) produced by A. *niger* (AN-004-75). Necrosis of the tubular cells in the corticomedullary and medullary zones of kidney [H.&E., x 100 (A) and x 400 (B)].



Lung was congested and had mild pneumonitis and thickening of the alveolar walls. Spleen was also congested. No significant changes were observed in other organs.

AN-010-75: Histopathologic examinations of various organs of weanling female rats died on tht first day after po administration of PES (0.9 ml/rat) produced by A. *niger* (AN-010-75).

Liver: Massive hemorrhagic necrosis of the hepatic cells was found in the centrolobular zones with pycnotic and karyorhetic nuclei, and cell debris. Adjacent to this zone, fatty degeneration of the hepatic cells was observed as large vacuoles which caused cytoplasm of these cells in the midzonal to periportal areas. The damage was accounted for nearly 90.0% of the liver (Fig. 2A and B).

Kidney: Severe necrosis of the epithelial cells in most convoluted tubules was observed throughout the kidney. Glomeruli were mostly congested and necrosis.

No significant changes were observed in the other organs.

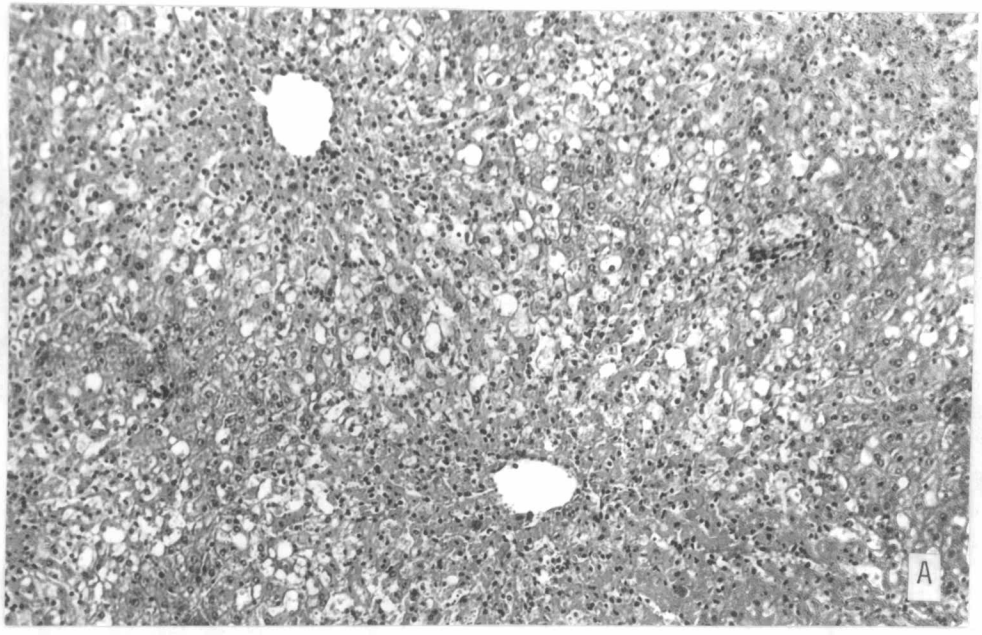
AN-008-75: Histopathologic examinations of various organs of weanling female rats died in the first day after ip administration of PEI (12.5 g moldy rice) produced by A. *niger*(AN-008-75).

Kidney: Necrosis of the epithelial in cells at the proximal and distal tubules was seen. There were two type of degenerative changes. First, necrosis of the tubules with pycnotic nuclei and second, degenerative changes with enlarged nuclei and desquamation of these cells into the tubular lumen. Congestion was also observed in the corticomedullary zone (Fig. 3A and B).

Lung was slightly congested.

Figure 2

Liver of weanling female rat died on the first day after po administration of PES (0.9 ml/rat) produced by *A. niger* (AN-010-75). Massive hemorrhagic necrosis of the hepatic cells in the centrolobular zones and fatty degeneration in most of midzonal areas of liver [H. & E., x100 (A) and x400 (B)].



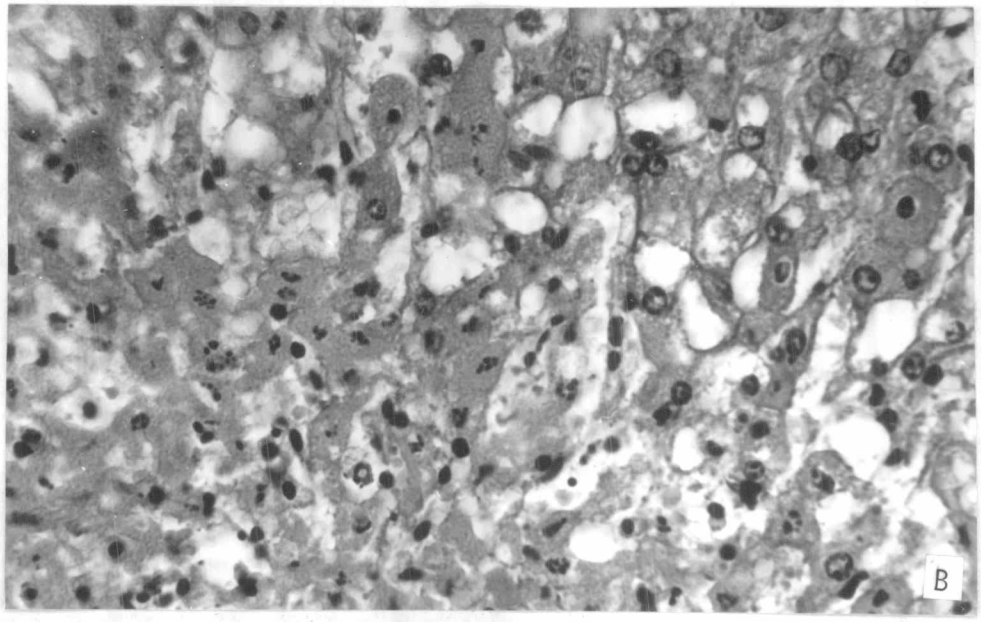
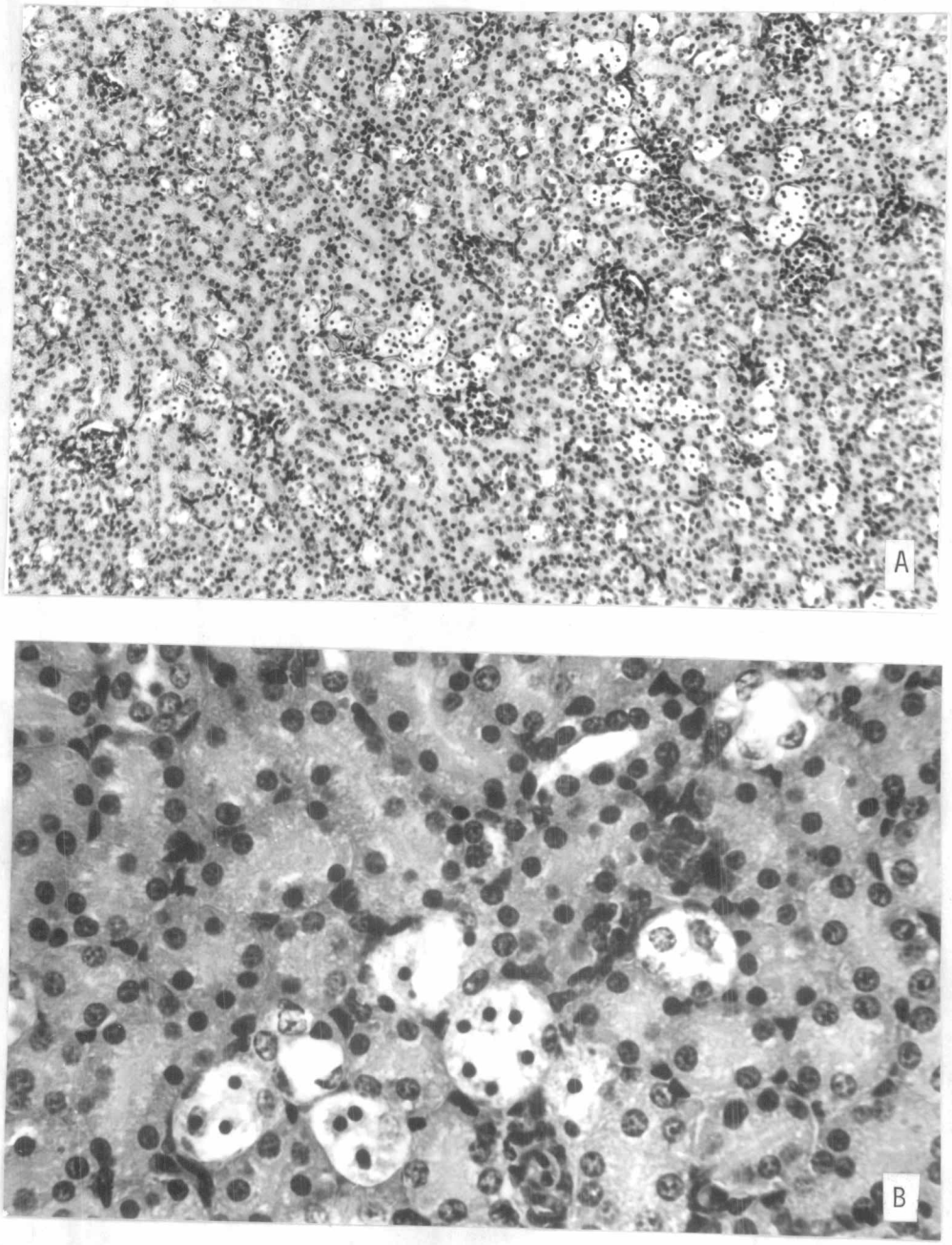


Figure 3

Kidney of weanling female rat died on the first day after ip administration of PEI (12.5 g moldy rice) produced by A. *niger* (AN-008-75). Necrosis of the epithelium in proximal and distal tubules in the cortex of kidney [H. & E., x100 (A) and x400 (B)].



AN-034-75: Histopathologic examinations of various organs of weanling female rats died in the first day after po administration of PES (1.0 ml/rat) produced by *A. niger* (AN-034-75).

Liver: Histopathologic changes revealed hepatic cells, Kupffer's cells and bile duct cells. Hepatic cells showed marked irregularity in the sizes of hepatic nuclei. Generalized dilatation of sinusoids throughout the liver was also seen with necrosis of the Kupffer's cells. Some of the Kupffer's cells picked up the sort of pigments. In addition, bile duct cells had gone into the necrotic processes with pycnotic nuclei and cell debris presented in the lumen of the ducts.

Kidney: Severe necrosis of the tubular epithelial cells in most of convoluted tubules was seen throughout the kidney. Most of the glomeruli were almost intact (Fig. 4A and B).

Spleen: Necrosis of spleen was seen at the edge of the organ with an increase in the number of the giant cells.

Other organs had no significant histopathologic changes.

AN-A30-75: Histopathologic examinations of various organs of weanling female rats died in the first day after ip administration of PEI (12.5 g moldy rice) or partial purified PEI (12.5 g moldy rice) produced by *A. niger* (AN-A30-75).

Liver: Only acidophilic cytoplasm was observed in some of the hepatic cells.

Kidney: Severe necrosis of the epithelium in most convoluted tubules was observed throughout the kidney. Congestion was found in the corticomedullary zones. No significance differences in the histopathologic changes were observed in the kidney of rats treated with

Figure 4

Kidney of weanling female rats died on the first day after po administration of PES (1.0 ml/rat) produced by A. *niger* (AN-034-75). Necrosis of the epithelium in most of convoluted tubules throughout the kidney [H. & E., x100 (A) and x400 (B)].

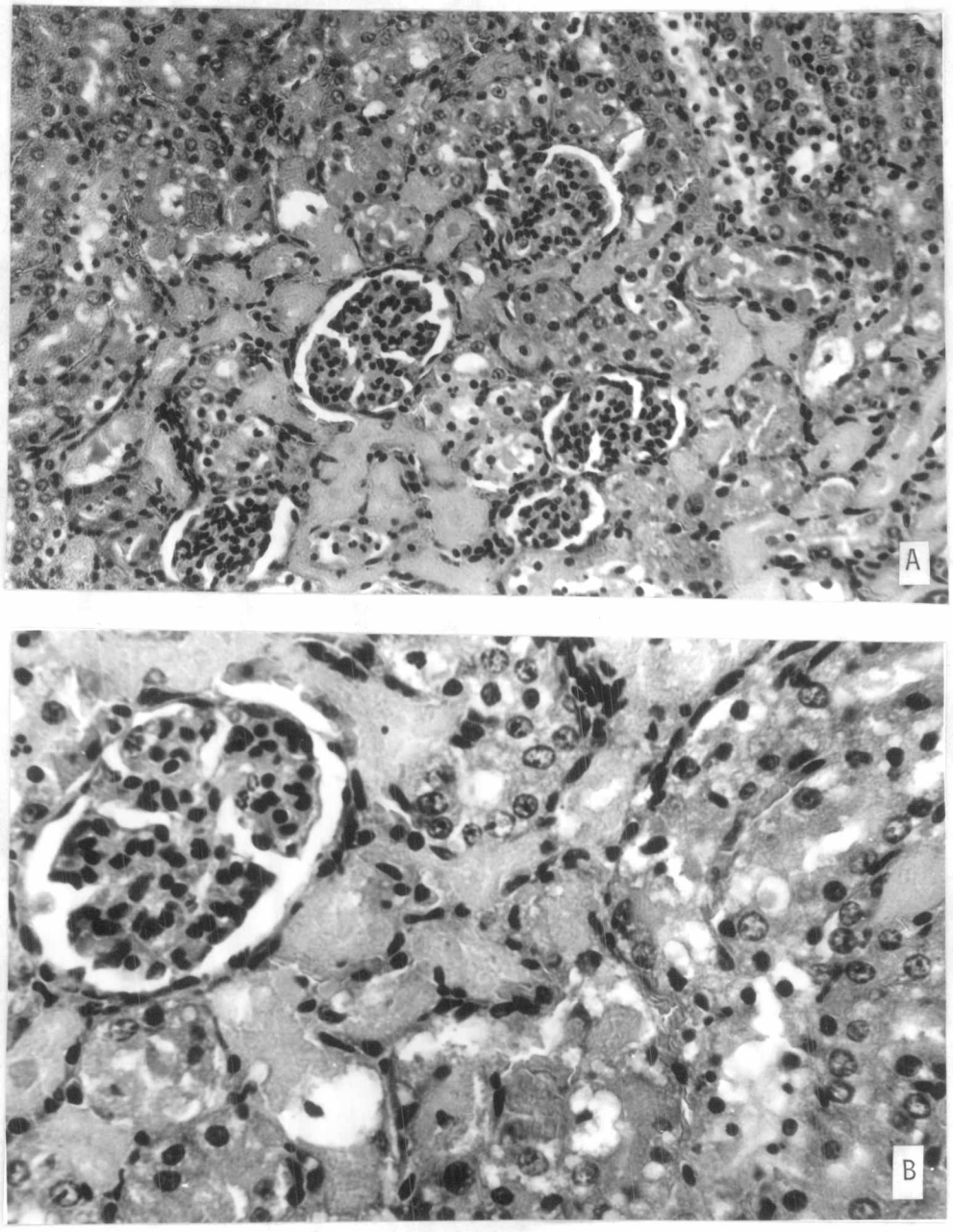
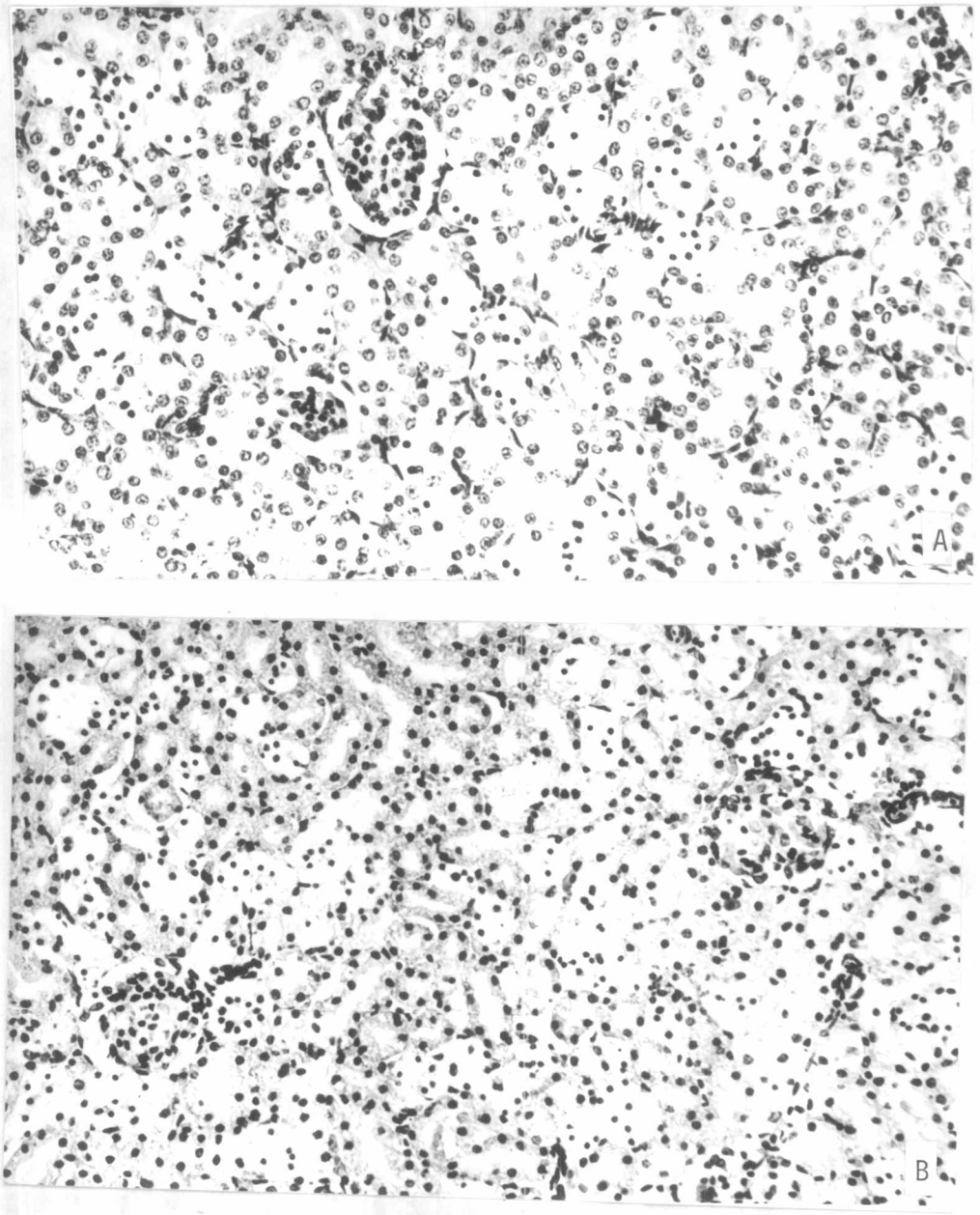


Figure 5

Kidney of weanling female rat died on the first day after ip administration of PEI (12.5 g moldy rice) or partial purified PEI (12.5 g moldy rice) produced by A. *niger* (AN-A30-75). Necrosis of the epithelium in most proximal and distal tubules of the cortex of kidney [H. & E., x100 (A PEI) and x100 (B partial purified)].



PEI or partial purified PEI (Fig. 5A and B).

No significant hitopathologic changes were observed in other organs.

AC-023-75: Histopathologic examinations of various organs of weanling female rats died in the first day after po administration of PES (0.40 ml/rat) produced by A. *candidus* (AC-023-75).

Liver: Histopathologic changes revealed severe necrotic degeneration of the hepatic cells in the centrolobular zones of liver. The necrotic area was extended to the midzonal area. Hepatic cells in this area were markedly enlarged containing pycnotic nuclei and degenerative cytoplasm. Some of the nuclei were swollen. Necrotic area was approximately 50.0% of the liver (Fig. 6A and B).

Kidney: Severe hemorrhagic necrosis of the epithelium of most convoluted tubules in the corticomedullary zones of kidney. The epithelial cells of most convoluted tubules were swollen with the enlarged vacuoles in the cytoplasm. These vacuoles seemed to generate from the basal membranes of the epithelium. Some of these cells were also sloughed off into the lumen (Fig. 7A and B).

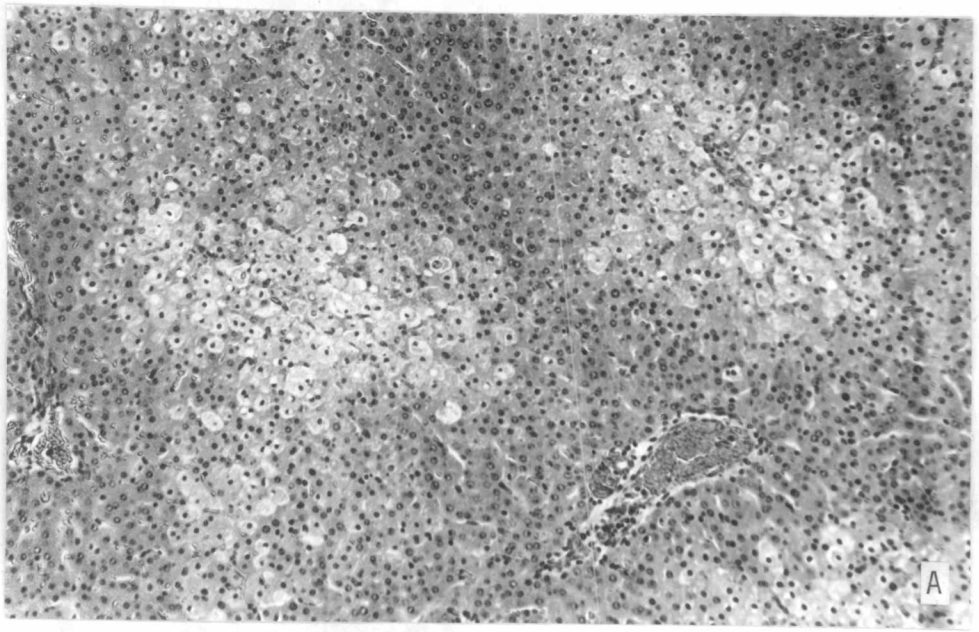
Spleen was congested. Lung was also severely congested with the thickening of the alveolar walls. No significant histopathologic changes were observed in other organs.

AFU-007-75: Histopathologic examinations of various organs of weanling female rats died in the second day after ip administration of PEI (12.5 g moldy rice) produced by A. *fumigatus* (AFU-007-75).

Kidney: Histopathologic changes found only in kidney revealed marked dilatation of most convoluted tubules in the cortex

Figure 6

Liver of weanling female rat died on the second day after po administration of PES (0.4 ml/rat) produced by A. *candidus* (AC-023-75). Massive centrolobular necrosis and the enlarged of the hepatic cells. Most of the nuclei of the hepatic cells in necrotic area were pycnotic [H. & E., x100 (A), and x400 (B)].



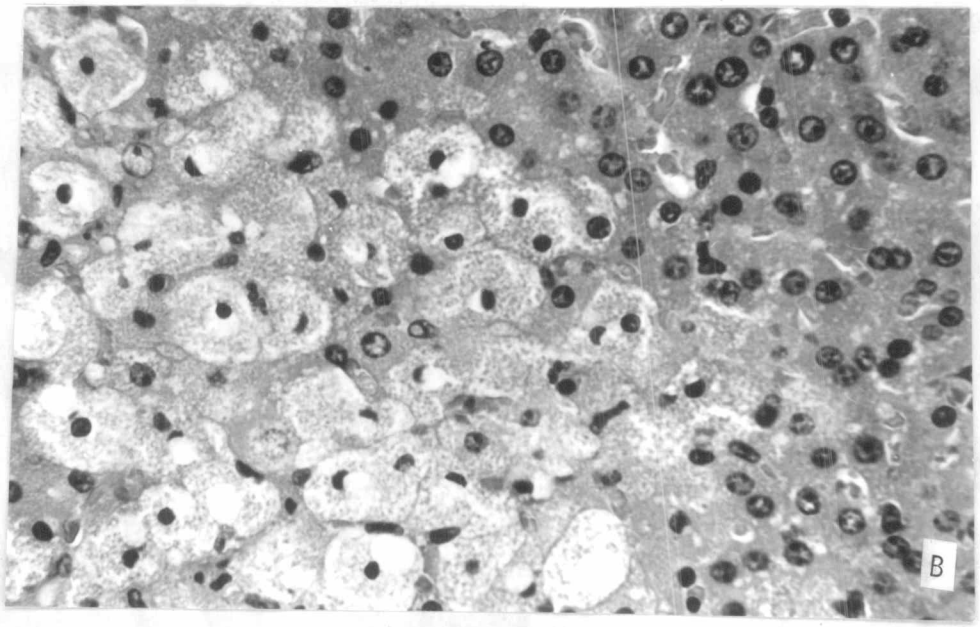
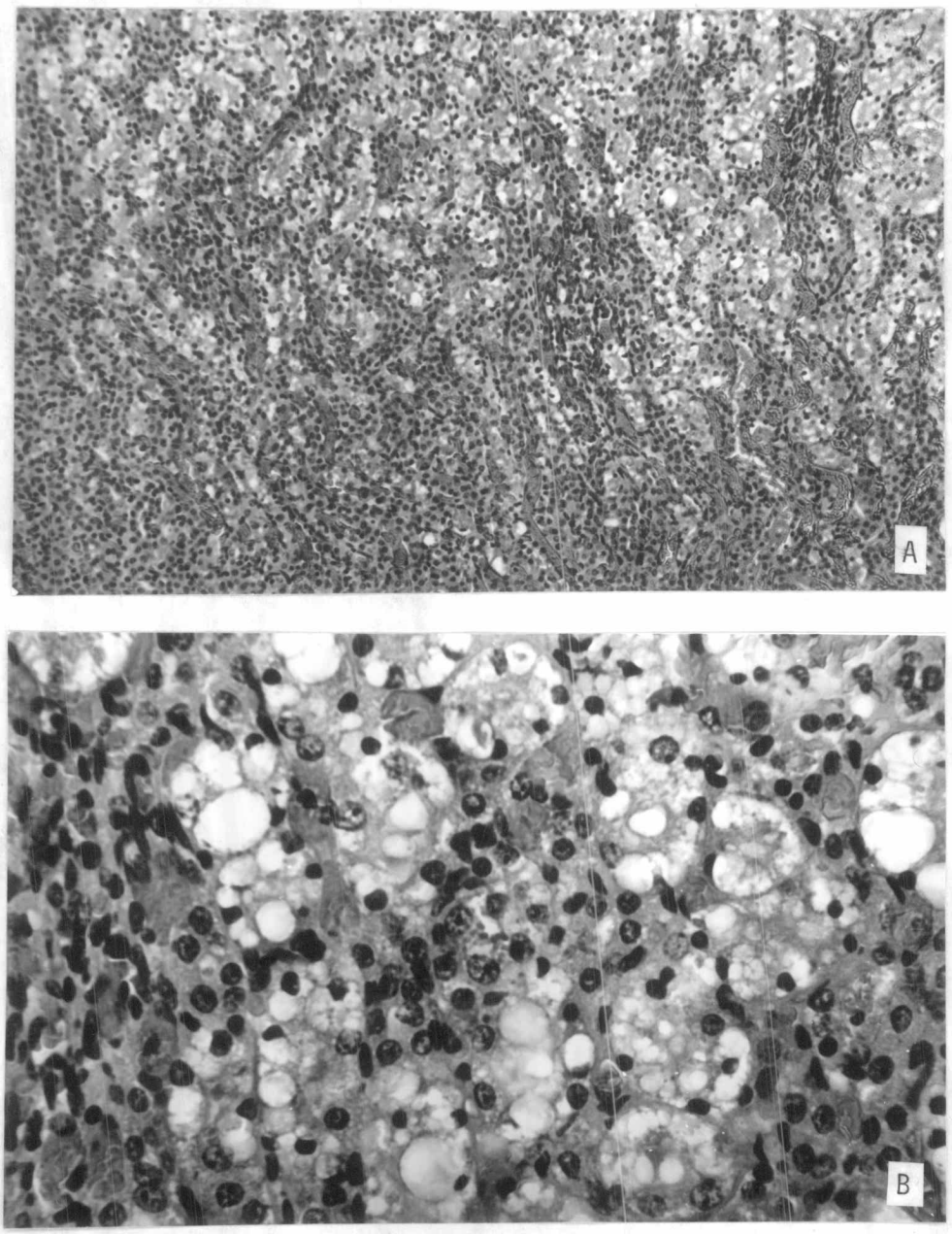


Figure 7

Kidney of weanling female rat died in the second day after po administration of PES (0.4 ml/rat) produced by A. *candidus* (AC-023-75). Severe necrosis with the large vacuoles of the epithelium in most convoluted tubules in the cortex and corticomedullary area of kidney [H. & E., x100 (A) and x400 (B)].



and corticomedullary zones.The epithelial cells were also flattened in these convoluted tubules. Necrosis of the tubular epithelial cells of the convoluted tubules were also observed in the cortico-medullary zones. These cells were also enlarged (Fig. 8A, B and C).

Lung: Congestion the alveolar sacs was observed in the lung. The alveolar walls were slightly dilated and thin.

No significant histopathologic changes were observed in the other organs.

AND-005-75: Histopathologic examinations of various organs of weanling female rats sacrificed on the seventh day after po administration of PES (1.0 ml/rat) produced by A. *nidulans* (AND-005-75).

Kidney: No serious histopathologic changes were observed in the tubular epithelial cells, except there was a lot of hyaline casts were seen in many convoluted tubules. These hyaline casts were also seen in the Bowman's capsules with slight necrosis in the glomeruli (Fig. 9A and B).

No significant histopathologic changes were observed in other organs.

Figure 8

Kidney of weanling female rat died on the first day after po administration of PES (1.0 ml/rat) produced by A. *fumigatus* (AFU-007-75). Marked dilatation of the convoluted tubules and Bowman's spaces of kidney [H. & E., x100 (A) and x400 (B)].

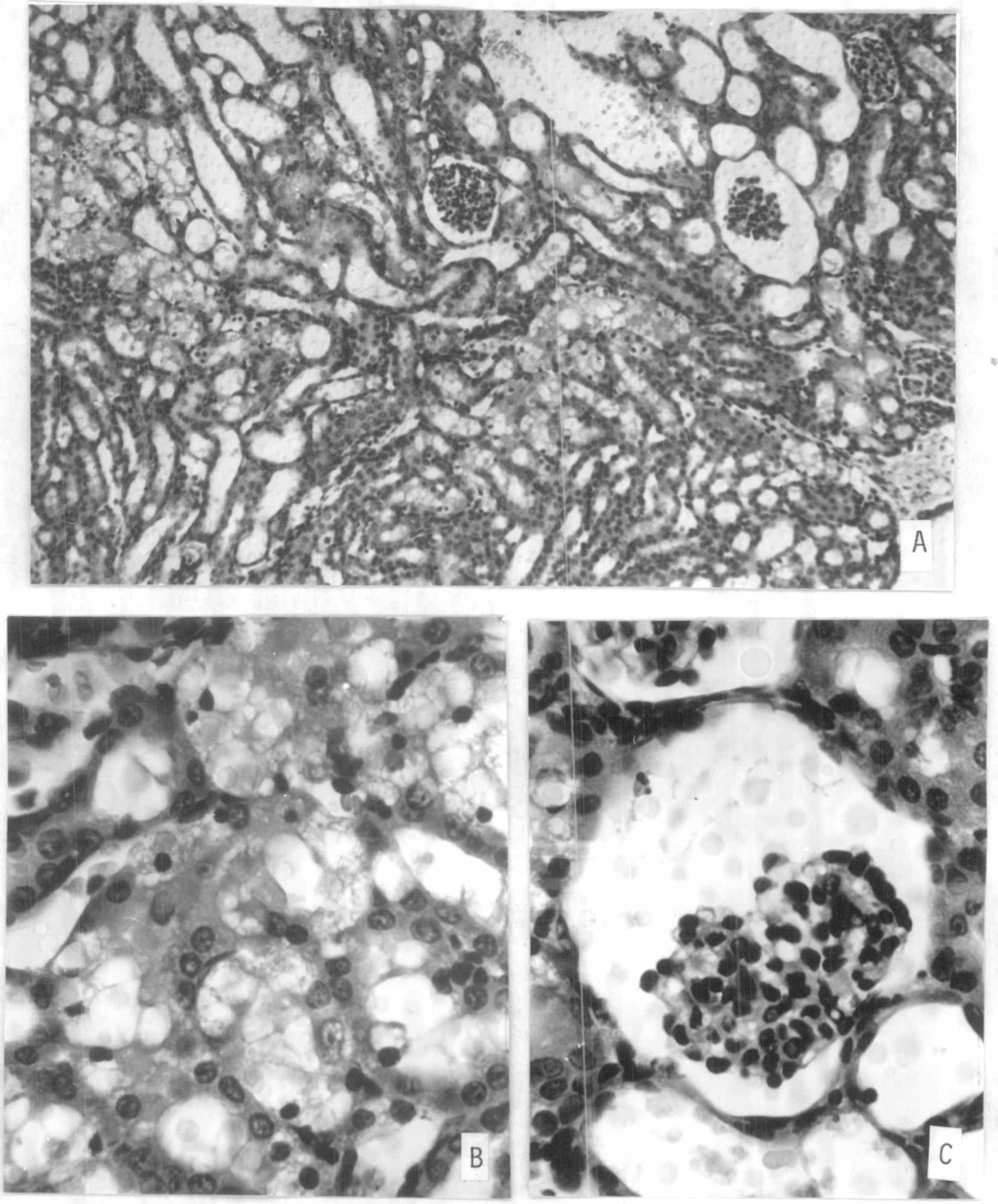
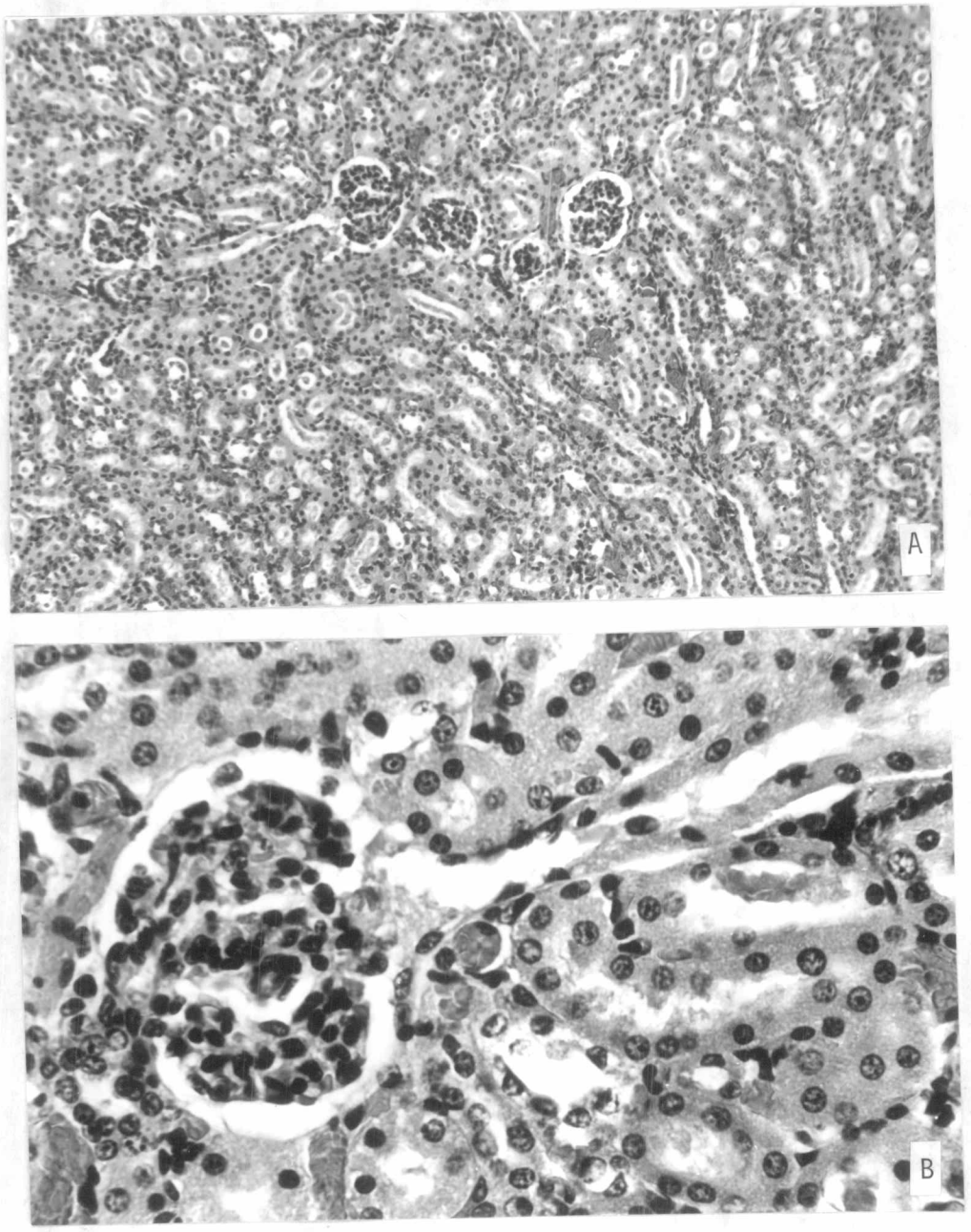


Figure 9

Kidney of weanling female rat sacrificed on the seventh day after po administration of PES (1.0 ml/rat) produced by A. *nidulans* (AND-005-75). Presence of the hyaline casts in the convoluted tubules of kidney [H. & E., x100 (A) and x400 (B)].



Production, isolation and purification of a mycotoxin from A. *niger* (AN-A30-75)

From one kilogram of moldy rice, 2.50 g crude toxin were obtained from this strain of A. *niger* (AN-A30-75). When crude toxin was fractionated by column chromatography using chloroform:benzene (50:50, v:v), chloroform:methanol (various concentrations) and methanol many of non-toxic compounds as shown in Fig. 10 was eluted in the first 11 fractions (825 ml). Most of these compounds were fluorescence in various colors. The total of the solid materials in these fraction were approximately 212 mg (47.6% of all solid materials recovered from column chromatography. The toxic fraction was eluted by chloroform-methanol mixtures in the fraction numbers 12 to 14 consisting of compound numbers 4, 7, 8 and yellow pigments. The fraction numbers 15 to 40 were found not to be toxic to 7-day old rats. After further purification it was found that compound number 8 (bright blue green fluorescence) was a toxic substance. The solid material of the compound number 8 was rather small of 2.4 mg. The comparable lethal toxicities of the crude toxin and compound number 8 were 15.0 mg/rat and 2.0 mg/rat respectively.

Acute toxicity of the crude toxin and potassium oxalate

Comparable acute toxicities of the crude toxin and potassium oxalate was studied in weanling female and the results are shown in Fig. 11. The crude toxin had LD_{50} of 560 mg/kg BW with the slope function of 1.192 and 508-618 mg/kg BW confidence limits (95%) whereas potassium oxalate had LD_{50} of 196 mg/kg BW with the slope function of 1.470 and 156-247 mg/kg BW confidence (95%). Potassium oxalate was approximate 3 times more toxic than the crude toxin in weanling female rats.

Figure 10. Compounds in crude toxin and various fractions of column eluates.

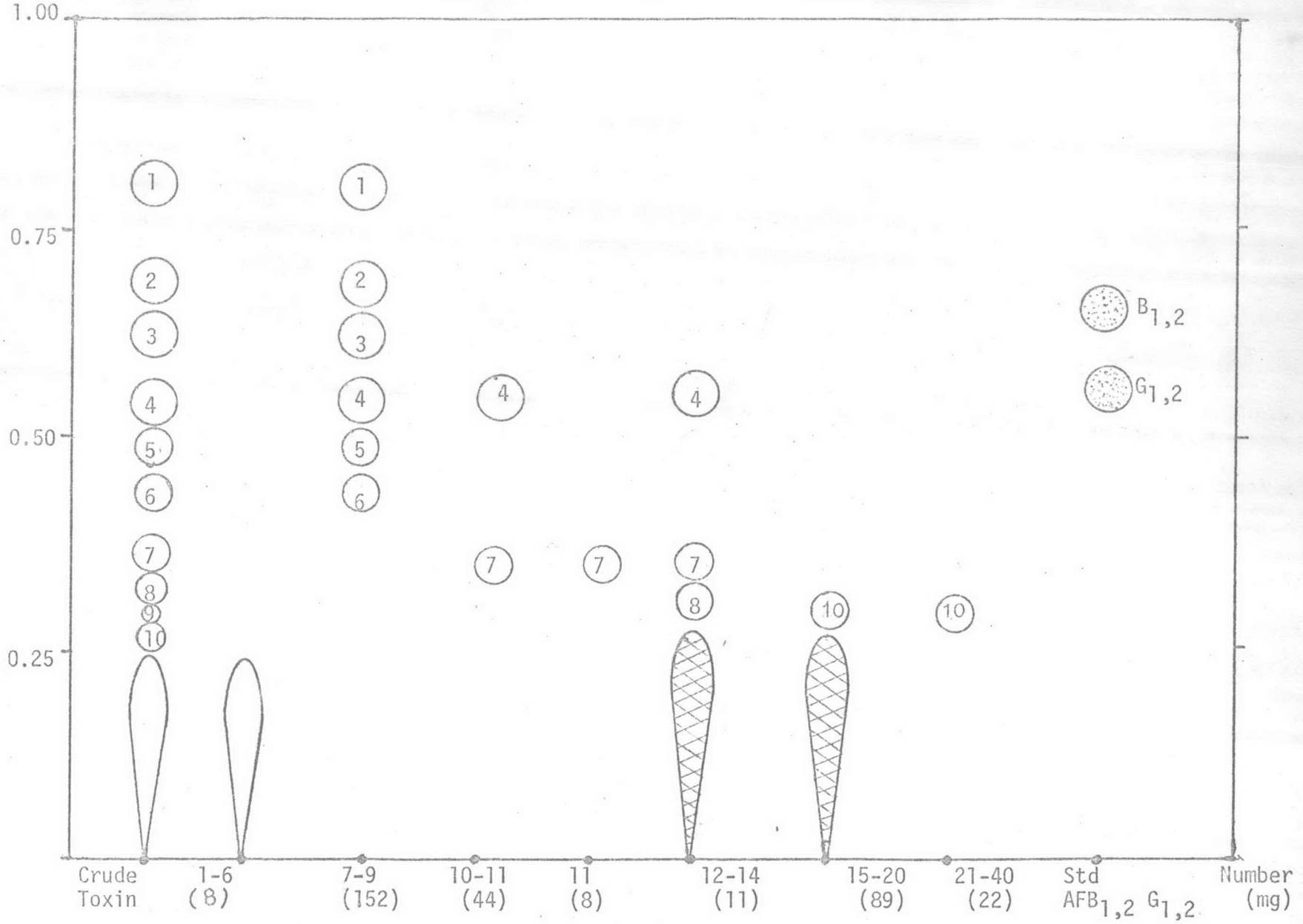


FIGURE10 Number of fractions of column eluates

Figure 10. Compound in crude toxin and various fraction of column eluates:

1	=	Yellow brown fluorescence, R_f value = 0.81
2	=	Yellow fluorescence, R_f value = 0.71
3	=	Yellow brown fluorescence, R_f value = 0.66
4	=	Dark yellow fluorescence, R_f value = 0.55
5	=	Yellow fluorescence, R_f value = 0.44
6	=	Yellow brown fluorescence, R_f value = 0.41
7	=	Dark blue fluorescence, R_f value = 0.36
8	=	Bright blue green fluorescence, R_f value = 0.32
9	=	Orange fluorescence, R_f value = 0.30
10	=	Green fluorescence, R_f value = 0.28
Std	=	Standard aflatoxin B and G.

Figure 11 LD_{50} of potassium oxalate and crude toxin from AN-A30-75