

# Studies on the Population of Toxigenic Fungi in Foodstuffs\*

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## ABSTRACT

These studies were carried out to detect the presence of mycotoxin producing fungi in various foodstuffs in Korea.

The experiments were divided into three parts: bacteriologic, toxicologic and electron microscopic studies.

From the 133 various samples, 425 colonies of fungi were isolated. In 405 of the 426 colonies it was possible to identify 17 genera. Among the identified strains the predominant genera were *Penicillium*, *Aspergillus* and *Alternaria*.

In the cytotoxicity test, 18 strains showed imld to severe toxic effects in mice, 19 strains showed toxic effects on HeLa cells.

In electron microscopic studies of liver cells from animals which had been treated with toxin-like substances, the liver cells showed the cytoplasmic changes dilatation of endoplasmic reticulum, swelling of mitochondria and increased number of lipid and glycogen particles. Alterations of nuclear envelope were also noted.

that are hepatotoxic, and carcinogenic, many investigators (Austwick and Ayerst, 1966; Boller and Schroeder, 1966; Diener, 1960; Kurata et al., 1968; Levi and Borker, 1968; Ueno et al., 1971; Christensen, 1957; Koh et al., 1971; Uraguchi et al., 1961; Carnaghan, 1965) have been intersted in mycotoxins. These problems have now a world-wide significance in terms of public health, agriculture, economics, etc., because cereals are one of the major foods in oriental countries.

In 1961, aflatoxin, a fungal metabolite with carcinogenic activity, was reported in England by Sargeant and others. Furthermore many other investigators (Diener, 1960; Coomes and Sanders, 1963; Lie and Marth, 1967; Scott, 1968; Masri et al., 1968; Jackson, 1968; Kurata et al., 1968; Mayer, 1969; Koh et al., 1973) have reported several mycotoxins isolated from various kinds of foodstuffs.

Several workers (Sargeants et al., 1961; Austwick and Ayerst, 1963; Blount, 1961; Asplin and Carnaghan, 1961; Enomoto and Saito, 1972) also have reported that aflatoxin has been shown to be the cause for the high incidence of hepatomas in some experimental animals (turkeys, poults, ducklings, chicks, sheeps, mice and rats). In addition, many mycotoxins for examples, ochratoxin, zearelenon, rubratoxin, fusarenon-X, leuteoskyrin, patulin, etc. have also been investigated as to their carcinogenic effect on the experimental

## INTRODUCTION

Since the discovery of fungal metabolites

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animals and cell cultures.

Furthermore, bioassay methods of mycotoxins have been reported, these make use of a cell culture system, histopathologic study of experimental animals, and chromatography of culture extracts. Kobayashi et al. (1958), Uraguchi et al. (1961), Saito et al. (1971), Salmon and Newberne (1963) and Koh et al. (1973) assayed mice, rats and cattles etc. Cell cultures, for example, human lung cells, liver cells, some strains of cell cultures, have also been used for mycotoxin assay (Gablick et al. 1965; Legator and Withrow, 1964; Zuckerman, 1967; Harley et al., 1969; Saito et al., 1971; Cho et al., 1973).

In the present paper, we summarized the classification of fungi isolated from various kinds of foods following the screening toxic strains using thin layer chromatography and bioassay of cultured cells and of mice. The electron microscopic observations of mice liver cells previously treated with culture extracts were also studied.

## MATERIALS AND METHODS

### A. Bacteriological Study

1. Sampling: A total of 133 bags of various kinds of foodstuffs were sampled from four geographical regions in Korea (Kyunggi-Do, Gangwon-Do, Kyungsang-Do and Seoul). All of the samples were collected between May of 1971 and June, 1972 (Table 1).

2. Isolation of Fungi: The samples were inoculated following surface washing with sterile distilled water and cultured on pepton-glucose agar plates containing 100mg per liter of chloramphenicol to suppress bacterial growth. Inoculated cultures were incubated

Table 1. Number and Kind of Samples

Kinds of Sample	Number
Soy bean	18
Red bean	12
Rice	8
Kidney bean	8
Millet	7
Barley	5
Maize	5
Wheat	4
Sesame	3
Rice cake	63
Total	133

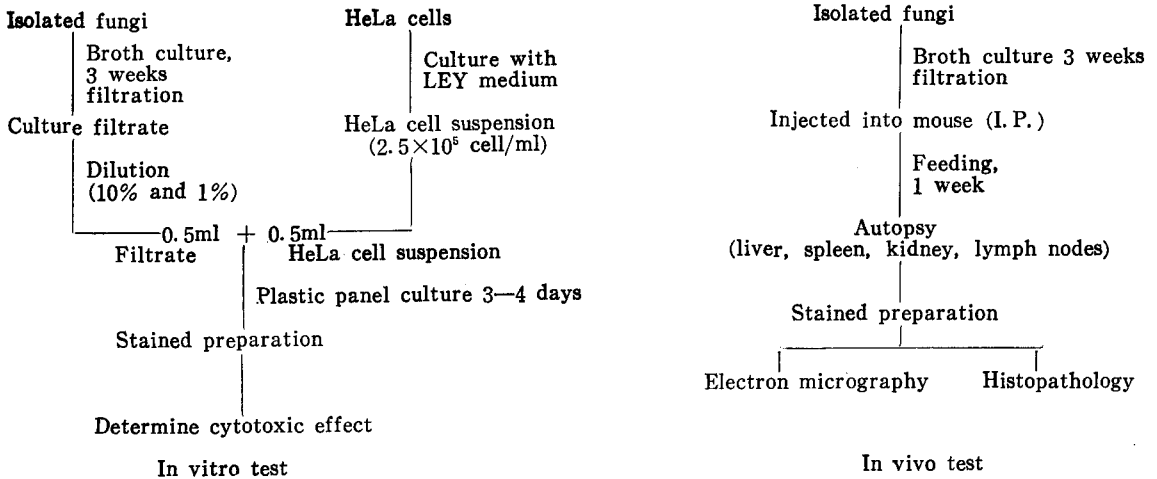
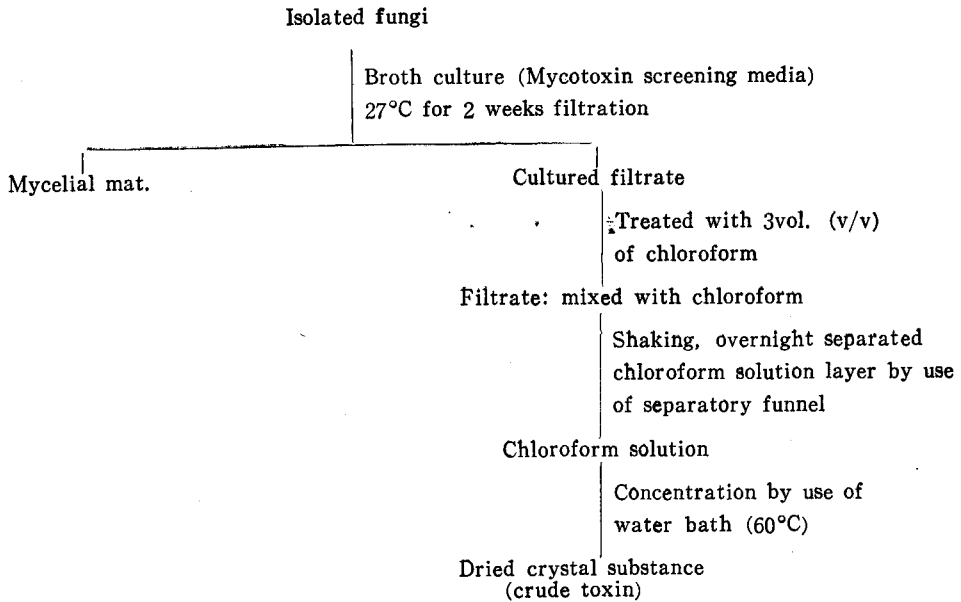
at 25°C and Observed daily. The growing colonies were transferred to potato-dextrose agar slants and cultured for identification.

3. Identification of fungi: *Aspergillus* and *Penicillium* strains were identified by consulting their descriptions (Raper and Thom, 1953; Raper and Fennell, 1965) for diagnosis and by the commonly employed spot and slide culture method using the Czapek's media, malt extract media, potato-glucose media and corn meal media.

### B. Toxicological Study

1. Crude toxin preparations: The crude toxin preparation procedure is schematically presented in Fig. 1. The fungal culture filtrate was mixed with chloroform and after being shaken overnight the chloroform layer was separated through a separation funnel. This chloroform solution was condensed at 60°C to an almost dried state (Fig. 1).

2. Toxicity screening on ICR-mice: ICR-mice, bred in our laboratory, were injected



intraperitoneally with a single dose of 1.0 ml/20gm body weight of the culture filtrates and sacrificed on the 5th day of the experiment. The animals were weighed everyday and major symptoms were recorded. Histopathological examination was carried out routinely on the liver, spleen, kidney, and lymph nodes (Fig. 2).

The degree of toxicity was graded as follows: 3+ for lethal toxicity, 2+ for the presence of definite pathologic lesions with decrease of body weight, 1+ for either definite pathological lesions or loss of body weight, and — for showing no positive evidence of any toxic effect.

### 3. Cytotoxicity test on HeLa cells: Cyto-

Table 2. Criteria for a Cytotoxic Rating System

Grade	Definition
0 :	Cells firmly adhering to the plastic with clear details and forming a sheet-like monolayer
1 :	Cell growth inhibited, but the majority of cells adhering to the plastic: some granulation and grouping of cells
2 :	Cells suspended and generally clumped with a pronounced granularity and the loss of cell detail; increasing cellular debris
3 :	Mainly single, suspended, shrunken cells with irregular membranes: some disintegrating cell clusters and considerable cellular debris.
4 :	Complete cytolysis: all debris

Table 3. Frequency of Occurrence of Fungal Species in 133 Samples

Name of Genera	Number	Percent(%)
Penicillium	115	27.0
Aspergillus	101	24.5
Alternaria	41	9.5
Mucor	39	9.2
Rhizopus	33	7.5
Fusarium	18	4.2
Cladosporium	14	3.2
Neurospora	12	2.6
Fusidium	6	1.3
Cepalotheicum	3	0.7
Syncephalostrum	2	0.5
Oedocephalum	2	0.5
Haplosporangium	2	0.5
Sepedonium	2	0.5
Absidia	2	0.5
Trichoderma	2	0.5
Yeast-like organisms	3	0.7
Unidentified	29	6.6
Total	426	100.0

toxicity of the culture filtrate was examined by the plastic panel technic of Toplin(1959) slightly modified as follows: LEY-medium

was supplemented with 10% calf serum and antibiotics. The culture filtrate was dissolved directly into the medium. The final concentration of the filtrates were 10% and 1%. Each cup of plastic panel which had been previously fixed with a cover glass received 0.5ml of HeLa cells suspension ( $1 \times 10^5$  cells/ml) and 0.5ml of the culture filtrates. After an incubation of 3 days at  $37^\circ\text{C}$  in a  $\text{CO}_2$ -incubator, the cells on the cover glasses were fixed with Carnoy's fixative and stained with hematoxylin-eosin. The cytotoxicity was classified by Toplin's criteria (Table 2).

### C. Electron Microscopic Study

For electron microscopy, specimens of the mouse liver cells were taken from the median lobe and fixed with 2% glutaraldehyde and 1% osmium tetroxide for 2 hours at  $4^\circ\text{C}$  in a veronal buffer at pH 7.4(Palade, 1952). All tissues were dehydrated in graded alcohol and embedded in Epon 812(Luft, 1961). Thin sections were cut with Porterblum Sorvall MT-2 ultramicrotome, stained with uranyl acetate and lead citrate solution, and observed under a Hitachi HU-11, E-1, type electron microscope.

### D. Control

1. Used strains: *Aspergillus flavus* ATCC

Table 4. Distribution of Predominant Fungal Species in Various Kinds of Sample

Name of Samples	Name of Predominant Genera
Soy bean :	Aspergillus, Penicillium, Rhizopus, Mucor, Neurospora, Alternaria, Sepedonium
Red bean :	Aspergillus, Penicillium, Rhizopus, Mucor Neurospora, Alternaria, Cladosporium, Trichoderma, Fusidium
Barley :	Aspergillus, Penicillium, Alternaria, Fusarium, Cladosporium
Sesame :	Aspergillus, Penicillium, Alternaria, Oedocephalum
Kidney bean:	Aspergillus, Penicillium, Mucor, Neurospora, Alternaria, Haplosporangium, Synccephalotrum
Wheat :	Aspergillus, Rhizopus, Mucor, Alternaria, Cladosporium
Millet :	Fusarium, Alternaria, Mucor, Rhizopus, Penicillium, Aspergillus, Cephalothecum
Rice :	Aspergillus, Penicillium, Alternaria, Cladosporium
Maize :	Aspergillus, Penicillium, Alternaria, Fusarium
Rice-cake :	Penicillium, Aspergillus, Rhizopus, Mucor

15517, *Aspergillus parasiticus* RIB 1037, *Aspergillus toxicarium* RIB 4002, *Aspergillus versicolor* IFO 4015, *Penicillium citrium* SWU 238, *Penicillium islandicum* IFO 5235, and *Penicillium tardum* IFO 5787 were used for controls in all of the experiments.

## RESULTS

A. Microflora isolated from various kinds of foodstuffs:

Of the 133 samples of foodstuffs, fungi were isolated from 98 samples, whereas 35 samples did not yield any fungi. Thus, fungi were isolated from 74.6 percent of the samples.

The genera and distribution of the fungi isolated are listed in Table 3 and 4. From the total 426 isolated strains recorded during examination, it was possible to identify 17 genera of fungi. It will be noted that *Penicillium* (27.0%), *Aspergillus* (24.5%) and *Alternaria* (9.5%) were the predominant genera.

B. Toxicity screening on HeLa cells:

The results of toxicity screening on HeLa

cells are summarized in Table 4.

Fungal isolates showing over 2+ toxicity were *Aspergillus* A-8, *Aspergillus* A-35, *Aspergillus* A-49, *Aspergillus* A-56, *Aspergillus* N<sub>1</sub>-7, *Penicillium* P-47, *Penicillium* P-63, *Alternaria* N<sub>1</sub>-11 and *Alternaria* C-10 (Table 5).

All of the control strains, *Aspergillus flavus* ATCC 15517, *Aspergillus parasiticus* RIB 1037, *Aspergillus toxicarium* RIB 4002, *Aspergillus versicolor* IFO 4105, *Penicillium citrium* SWU 238 and *Penicillium islandicum* IFO 5235 showed over 2+ toxicity grade (Table 6).

C. Toxicity screening of ICR-mice:

The results are summarized in Table 6. Twenty of the 42 tested strains showed mild to severe toxic effects on ICR-mice with culture filtrates. *Penicillium* P-32, *Penicillium* P-56, *Penicillium* P-66, *Penicillium* P-67, *Penicillium* N<sub>1</sub>-4, *Penicillium* N<sub>1</sub>-12, *Penicillium* RC-8, *Penicillium* P-75, *Aspergillus* A-35, *Aspergillus* N<sub>1</sub>-7, and *Alternaria* C-10 showed mild toxic effects and *Aspergillus* A-8, *Aspergillus* A-49, *Aspergillus* A-66,

**Table 5. Results of Toxicity Test on HeLa Cells in the Experimental Strains**

Name of Strains	Grade of Toxicity on HeLa cells	
	10% F*	1%F*
Aspergillus A-8	2	1
A-35	2	1
A-49	3	2
A-66	2	1
N <sub>1</sub> -25-5	0	0
N <sub>1</sub> -2	1	0
N <sub>1</sub> -7	2	1
N <sub>1</sub> -25-1	0	0
N-25-2	1	1
Penicillium P-11	0	0
P-31	0	0
P-32	1	0
P-33	0	0
P-35	0	0
P-36	0	0
P-39	0	0
P-44	0	0
P-47	2	1
P-49	0	0
P-50	0	0
P-54	0	0
P-56	1	0
P-61	0	0
P-63	2	0
P-66	1	0
P-67	1	0
P-75	0	0
N <sub>2</sub> -1	0	0
N <sub>1</sub> -2	0	0
N <sub>1</sub> -3	1	0
N <sub>1</sub> -9	0	0
N <sub>1</sub> -10	0	0
N <sub>1</sub> -11	0	0
N <sub>1</sub> -12	1	0
N <sub>1</sub> -14	0	0
N-19	0	0
RC-8	1	0
RC-10	0	0
RC-11	0	0
RC-18	0	0
Alternaria N <sub>1</sub> -11	2	1
C-10	1	1

\* F : Culture filtrate

**Table 6. Results of Toxicity Test on HeLa Cells in the Referencen Strains**

Generic Name	Grade of Toxicity on HeLa Cells	
	10%F*	1%F*
Aspergillus flavus ATCC 15517	3	3
Aspergillus parasiticus RIB 1037	3	2
Aspergillus toxicarium RIB 4002	2	1
Aspergillus versicolor IFO 4105	2	2
Penicillium citrium SWU 238	3	2
Penicillium islandicum IFO 5235	2	1
Penicillium tardum IFO 5787	1	0
Test Control	0	0
Control	0	0

\* F : Culture filtrates

Penicillium P-47, Penicillium P-63 showed severe toxic effects (Table 7).

The control strains, Aspergillus toxicarium RIB 4002, Penicillium citrium SWU 238, Penicillium islandicum IFO 5235 showed mild toxic effects but Aspergillus flavus ATCC 15517, Aspergillus parasiticus RIB 1037 and Aspergillus versicolor IFO 4105 showed severe toxic effects (Table 8).

The histopathological toxic patterns of organ damage induced by the toxic strains were almost all hepatotoxic pattern: centrolobular or single cell necrosis, fatty and vacuolar degeneration of parenchymal cells, and slight or marked body weight loss.

#### D. Electron microscopy:

These experiments were proceeded others which used several mice which had been treated with toxic producing fungal strains.

Four of the 18 experimental strains(Penicillium N<sub>1</sub>-12, Penicillium P-32, Penicillium

Table 7. Results of Toxicity Tests on the ICR-Mice in the Experimental Strains

Name of Strains		Grade of the Toxicity on the ICR-Mice
Aspergillus	A-8	2+
	A-35	1+
	A-49	3+
	A-66	2+
	N <sub>1</sub> -25-5	—
	N <sub>1</sub> -2	—
	N <sub>1</sub> -7	1+
	N <sub>1</sub> -25-1	—
	N <sub>1</sub> -25-2	1+
Penicillium	P-11	—
	P-31	—
	P-32	1+
	P-33	—
	P-35	—
	P-36	—
	P-39	—
	P-44	—
	P-47	3+
	P-49	—
	P-50	—
	P-54	—
	P-56	1+
	P-61	—
	P-63	3+
	P-66	1+
	P-67	1+
	P-75	1+
	N <sub>2</sub> -1	—
	N <sub>1</sub> -2	1+
	N <sub>1</sub> -3	1+
	N <sub>1</sub> -9	—
	N <sub>1</sub> -10	—
	N <sub>1</sub> -11	—
	N <sub>1</sub> -12	1+
	N <sub>1</sub> -14	—
	RC-8	1+
	RC-10	—
	RC-11	—
	RC-18	—
Alternaria	N <sub>1</sub> -11	1+
	C-10	1+

Table 8. Results of Toxicity Tests on ICR-Mice in the Reference Strains

Generic Name	Grade of Toxicity on ICR-Mice
Aspergillus flavus ATCC 15517	2+
Aspergillus parasiticus RIB 1037	3+
Aspergillus toxicarium RIB 4002	1+
Aspergillus versicolor IFO 4105	2+
Renicillium citrium SWU 238	1+
Penicillium islandicum IFO 5235	1+
Penicillium tardum IFO 5787	0
Control	0

N<sub>1</sub>-3 and Penicillium P-56) showed on irregular nuclear envelope and especially Penicillium P-32 showed nuclear inclusion bodies in the ICR-mice liver cells.

Penicillium P-32, Penicillium P-49, Penicillium P-61, Penicillium P-67, Penicillium N<sub>1</sub>-3, Penicillium N<sub>1</sub>-14, Aspergillus A-35, and Aspergillus A-66 showed mild dilatation or vacuolization of rough endoplasmic reticulum (RER). Penicillium P-56, Penicillium P-63, Aspergillus A-49, and Aspergillus N<sub>1</sub>-25-2 showed swelling of the mitochondria and disappearance of mitochondria cristae on the experimental mice liver cells. Lysosome and ribosome alteration were shown in mice treated with Penicillium N<sub>1</sub>-7, Penicillium N<sub>1</sub>-12 and Aspergillus A-6. Seven strains of experimental mice showed heavy deposits of glycogen and lipid particles in the liver cells (Electron microscopic photo. 6, 7, 8, 9, and 10).

All of the control strains showed cytoplasmic changes such as dilatation of rough endoplasmic reticulum (RER), swelling of mitochondria, detachment of ribosomes, heavily deposits of lipid droplets and glycogens (Electron microscopic photo. 2, 3, 4 and 5).

## DISCUSSION

Various cereals invaded by fungi cause several both acute or chronic disease in people living in districts where both people and animals use various grains as a foodstuffs. As for the causatives of the intoxication, numerous investigators (Diener, 1960; Coomes and Sanders, 1963; Lie and Marth, 1967; Scott, 1968; Masri et al., 1968; Brown et al., 1968; Mayer, 1969; Kurata et al., 1968; Scott, 1969; Pons, 1969; Koh et al., 1970) have pointed out that the intake of fungal metabolites is one of the major causes of intoxication.

According to previous data, Kurata and Ichinoe (1967), Kurata et al. (1968) have pointed out that the predominant strains contaminating flour type foodstuffs and rice in Japan were *Penicillium*, *Aspergillus*, and *Cladosporium* (among 100 samples of rice), Christensen et al. (1954; 1957; 1960; 1964) Garren (1966), Wallace and Smith (1968), Nobel et al. (1958) and Koh and Lew (1970) also noted that 17-22 genera have been in various kinds of foodstuffs. Predominant genera were *Penicillium*, *Aspergillus*, *Alternaria* and *Cladosporium*. The previous data coincides with our experimental results.

Toxicity screening of mycotoxins using a cell culture system and animals one of the feasible methods of mass screening of mycotoxin producing fungi. Saito et al. (1971; HeLa cell), Zuckerman (1968; human embryo liver cell), Dilimpio (1968; human leukocyte), Promachainant (1972; human leukocyte) Svoboda et al. (1964; monkey liver cell) and Cho et al. (1973; HeLa cell) used culture systems to detect the mycotoxin producing

fungi. They have asserted that these methods are valuable in the detections of mycotoxins. In our experiments 8 strains of the 44 tested strain, *Aspergillus* A-8, *Aspergillus* A-35, *Aspergillus* A-49, *Aspergillus* A-66, *Aspergillus* N<sub>1</sub>-7, *Penicillium* P-63, *Penicillium* P-47 and *Alternaria* N<sub>1</sub>-11 showed over 2+ toxicity on HeLa cells. 18 strains showed mild (*Penicillium* P-32, *Penicillium* P-56, *Penicillium* P-67, *Penicillium* N<sub>1</sub>-3, *Penicillium* RC-8, *Penicillium* P-75, *Aspergillus* A-35, *Aspergillus* N<sub>1</sub>-3 and *Alternaria* C-10) to severe (*Aspergillus* A-8, *Aspergillus* A-49, *Aspergillus* A-47, *Aspergillus* A-66, *Aspergillus* A-35, *Aspergillus* N-25-2, *Penicillium* P-47, *Penicillium* P-63 and *Penicillium* P-63 and *Penicillium* P-32) toxic effect on ICR-mice.

The cell culture system and the use of animal experiments are parallel screening methods for the detection of cytotoxic fungal metabolites. Comparing the results obtained with both of these methods one notes a similarity. Both revealed toxic effects in the samples which were tested in the most of the cases. However, in several fungal strains, cytotoxicity was not noted. On the contrary, no lethal effect was found in the animals treated with the samples. This finding may be due to activation or inactivation of the toxic metabolites within the body. Saito et al. (1971), Koh et al. (1974), Cho et al. (1973) and Smith and others (1963) have carried out cytotoxicity comparison studies in vitro and in vivo using fungal metabolites and chemicals. A statistically significant correlation was shown to exist between the two states. But, the correlations were not absolutely constant. Some of the samples which had severe toxicity in vitro had less

toxicity in animals. However, we considered it appropriate, for a first step screening of carcinogenic mycotoxins, to check the cytostatic or cytotoxic activity of metabolites of the test fungi.

Also, the electron microscopic study revealed that *Penicillium* N<sub>1</sub>-14, *Penicillium* P-32, *Penicillium* N<sub>1</sub>-3 and *Penicillium* P-61 were associated with irregular nuclear envelope and especially *Penicillium* P-32 produced a nuclear inclusion body inside the nucleus. Eight of the 18 tested strains caused dilatation or vacuolization with rough endoplasmic reticulum. 4 strains were associated with mitochondrial swelling or disappearance of cristae (*Penicillium* P-56, *Penicillium* P-63, *Aspergillus* A-49 and *Aspergillus* N-25-2). Lysosome and ribosomal changes were also observed following treatment of *Penicillium* P-47, *Penicillium* N<sub>1</sub>-7, *Penicillium* N<sub>1</sub>-12 and *Aspergillus* A-6 culture filtrates. Some liver cells showed heavy deposits of glycogen and lipid particles after addition of *Penicillium* P-32, *Penicillium* P-66 and *Penicillium* P-6. Kim (1971), Svoboda et al. (1968), Butler (1966) observed cytoplasmic changes such as dilatation of rough endoplasmic reticulum with detachment of ribosome, increased number of lipid and glycogen particles, nuclear envelope alterations and nucleolus capping phenomenon. These phenomenon agree with the findings other investigators (Kim, 1971; Svoboda et al. 1966; Wood, 1965; Sporn et al., 1966; Deung et al., 1973; 1974).

Based on the results of these experiments, several samples have been selected for further study to find the correlation between toxicity and carcinogenic effects. These studies also include the chemical isolation of metabolites, species identification of a fungi producing a

toxyn-like substance, chromosomal analysis of the cultured cells which have been exposed to metabolites, tests for teratogenic effects, and for some environmental factors affecting the metabolites.

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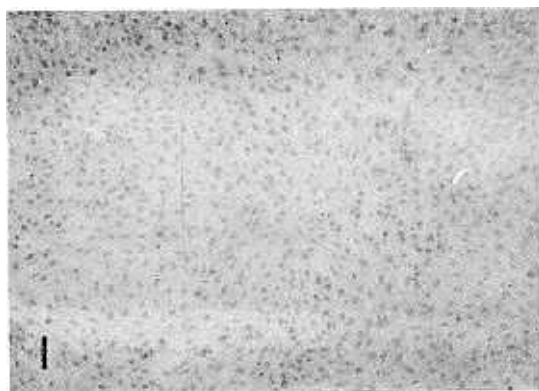


Fig. 1. Normal cultured HeLa cells.

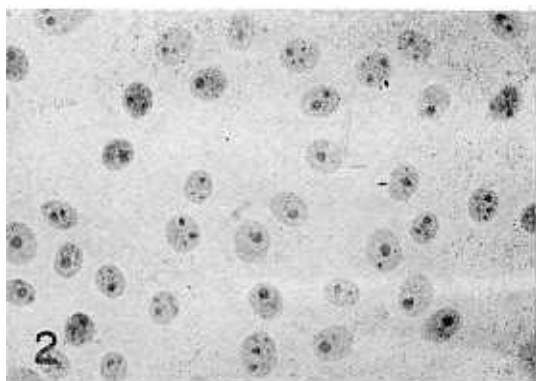


Fig. 2. Normal cultured HeLa cells(High powered magnification).

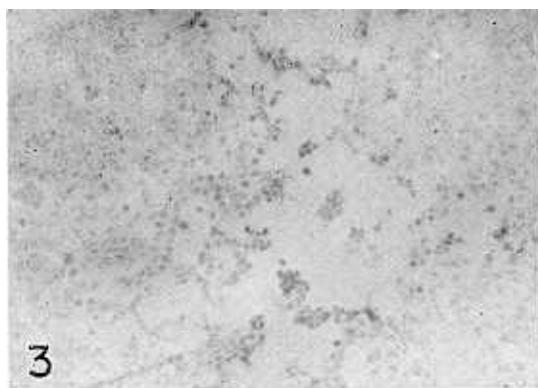


Fig. 3. Cytotoxicity grade 3 on HeLa cells treated with reference strain of *Aspergillus flavus* ATCC 15517.

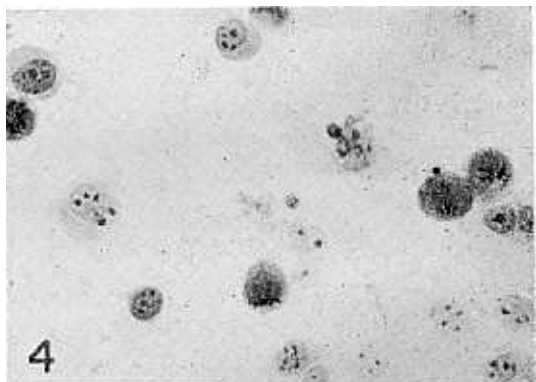


Fig. 4. Cytotoxicity grade 3 on HeLa cells treated with reference strain of *Aspergillus flavus* ATCC 15517 (High powered magnification).

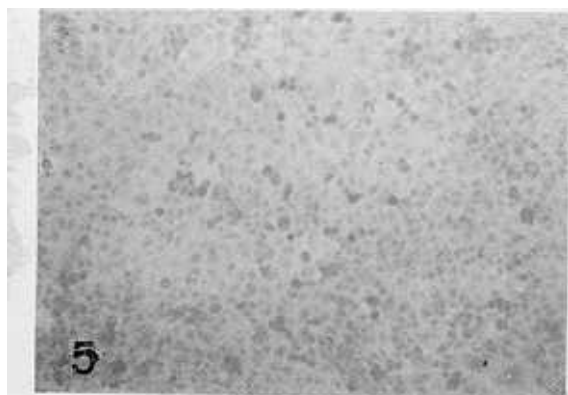


Fig. 5. Cytotoxicity grade 2 on HeLa cells treated with experimental strain of *Aspergillus* A-8.

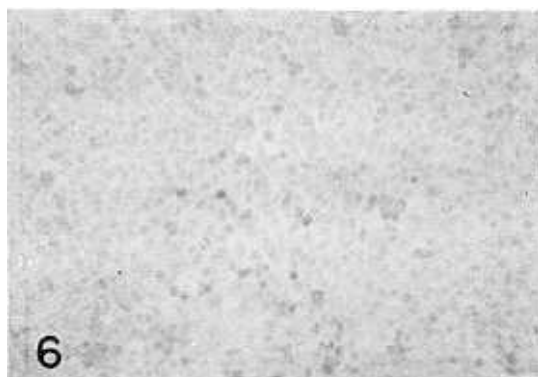


Fig. 6. Cytotoxicity grade 2 on HeLa cells treated with experimental strain of *Penicillium* P-47.

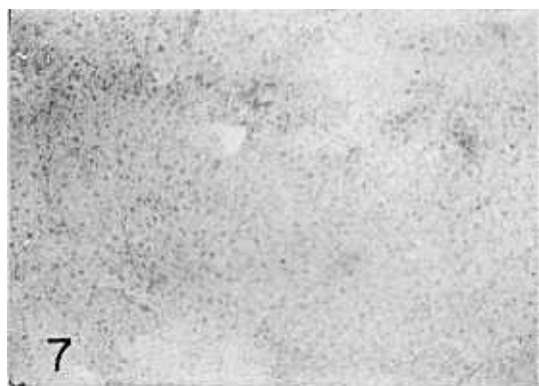


Fig. 7. Focal necrosis of mouse liver cells treated with reference strain of *Aspergillus parasiticus* RIB 1037.

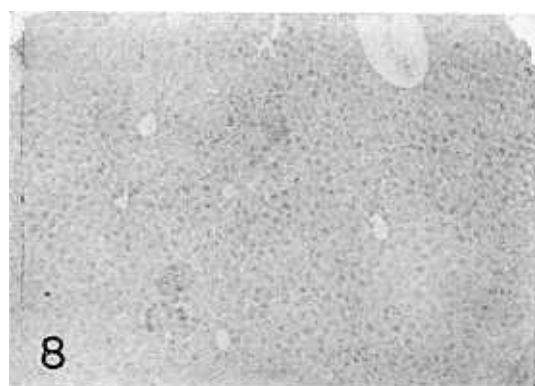


Fig. 8. Acute inflammatory changes of mouse liver cells treated with experimental strain of *Penicillium* P-32.

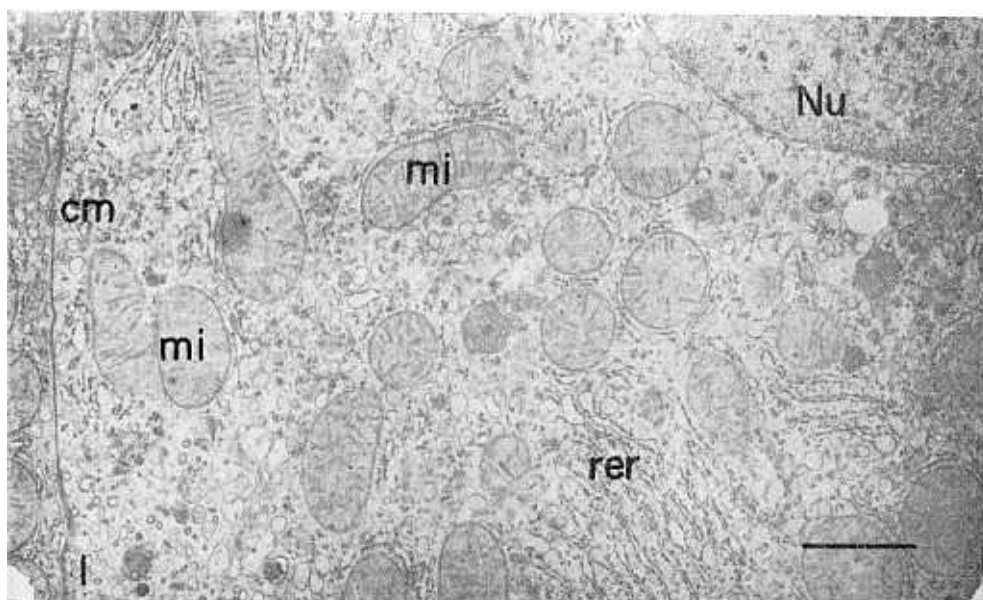


Fig. 1. Ultrastructure of normal mouse liver cell. Nu: nucleus, mi: mitochondria, rer: rough endoplasmic reticulum cm: cell membrane.

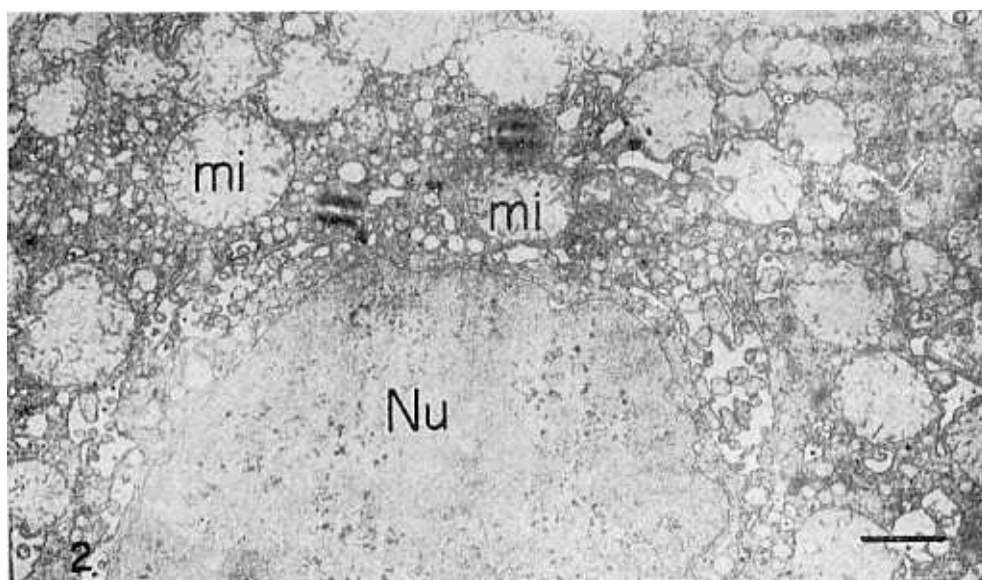
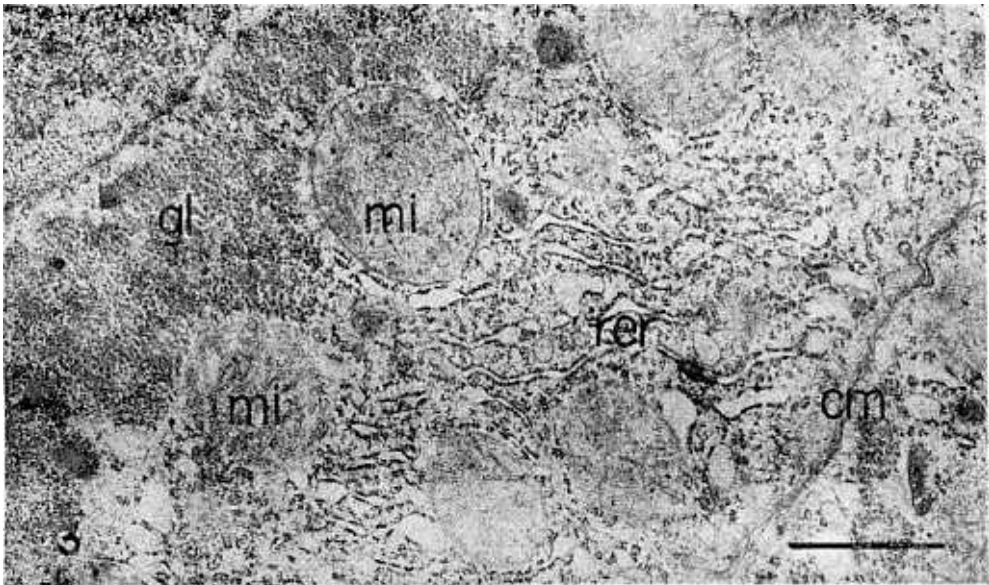
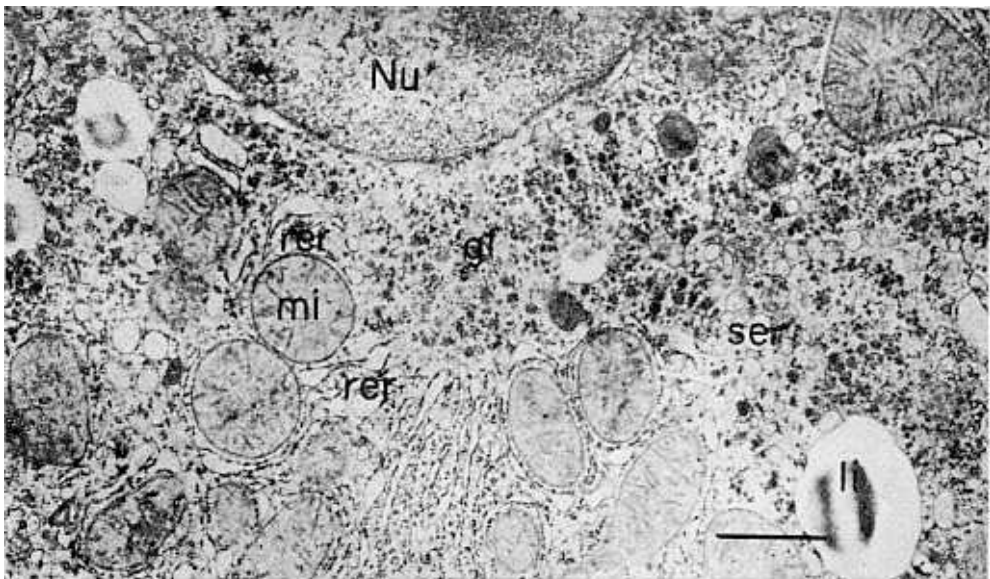


Fig. 2. Irregularity of mitochondrial membrane and disappearance of cristae (Reference Strain: *P. citrium* SWU 238). Nu:nucleus, mi:mitochondria.



**Fig. 3.** Heavy deposits of glycogen and slightly dilatation of rough endoplasmic reticulum were seen (Reference Strain: *P. islandicum* IFO 5235). mi: mitochondria, gl: glycogen, rer: rough endoplasmic reticulum, cm: cell membrane.



**Fig. 4.** Dilatation of rough endoplasmic reticulum, increased number of glycogen and lipid droplet were observed (Reference Strain: *A. flavus* ATCC 15517). Nu: nucleus, rer: rough endoplasmic reticulum, gl: glycogen, mi: mitochondria, ser: smooth endoplasmic reticulum, li: lipid droplet.

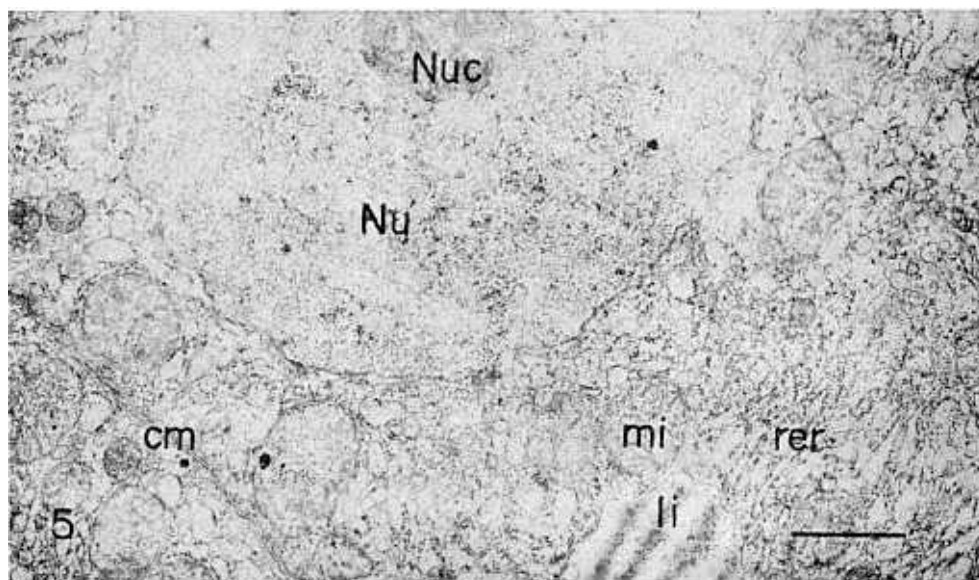


Fig. 5. Irregularity of the nuclear envelope, dilatation of rough endoplasmic reticulum and disappearance of mitochondrial cristae were also noted (Reference Strain: *P. islandicum* IFO 5235). Nuc: nucleolus, li: lipid droplet, cm: cell membrane, Nu: nucleus, rer: rough endoplasmic reticulum.

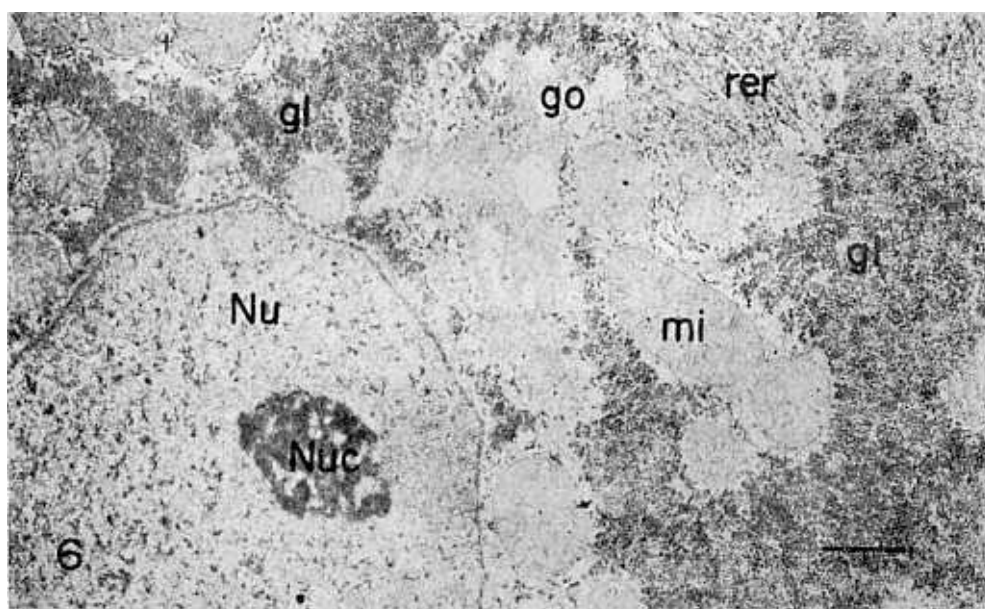


Fig. 6. Heavy deposits of glycogen and slightly rough endoplasmic reticulum dilatation were seen treated with culture filtrate of Experimental strain of *Penicillium* P-63. Nu: nucleus, go: golgi complex, gl: glycogen, mi: mitochondria, rer: rough endoplasmic reticulum.

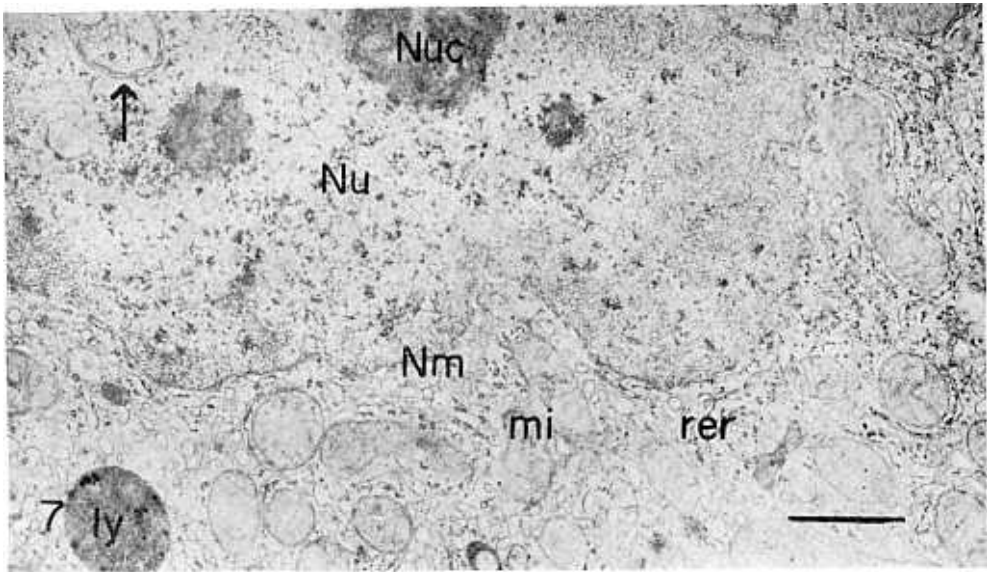


Fig. 7. Appearance of lysosome, irregularity of nuclear envelope and nuclear inclusion body were observed (Experimental strain of *Penicillium P-32*). ly: lysosome, Nm:nuclear envelope,  $\leftarrow$ : nuclear inclusion body.

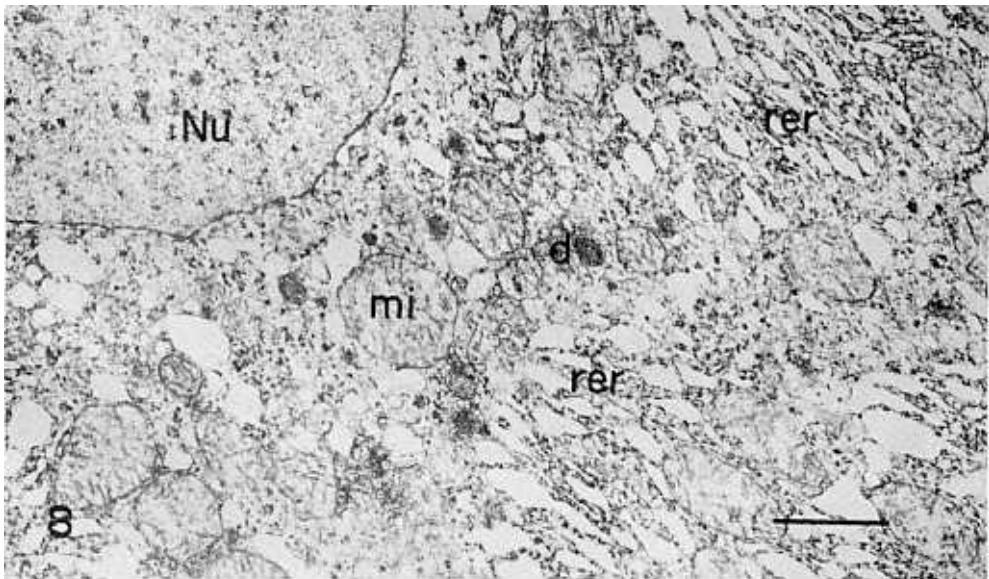


Fig. 8. Intensive changes of rough endoplasmic reticulum, disappearance of mitochondrial cristae and wrinkled mitochondrial membrane were observed (Experimental strain of *Aspergillus A-49*). Nu: nucleus, mi: mitochondria, rer:rough endoplasmic reticulum, d: dense body.

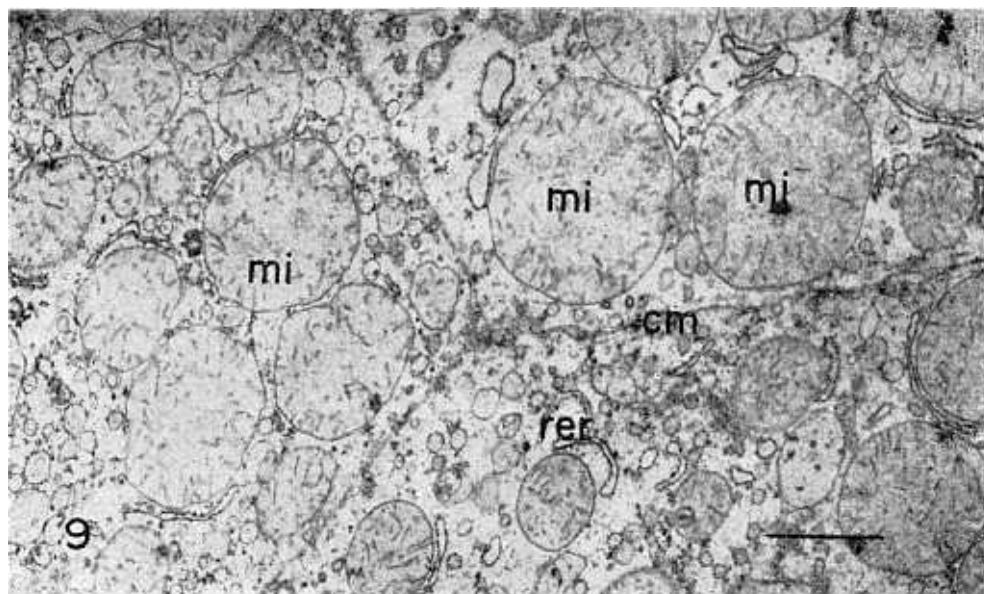


Fig. 9. Disappearance of mitochondrial cristae and swelling of mitochondria were also noted treated with culture filtrate of Experimental strain of *Aspergillus* A-49. mi: mitochondria, cm: cell membrane, rer: rough endoplasmic reticulum.

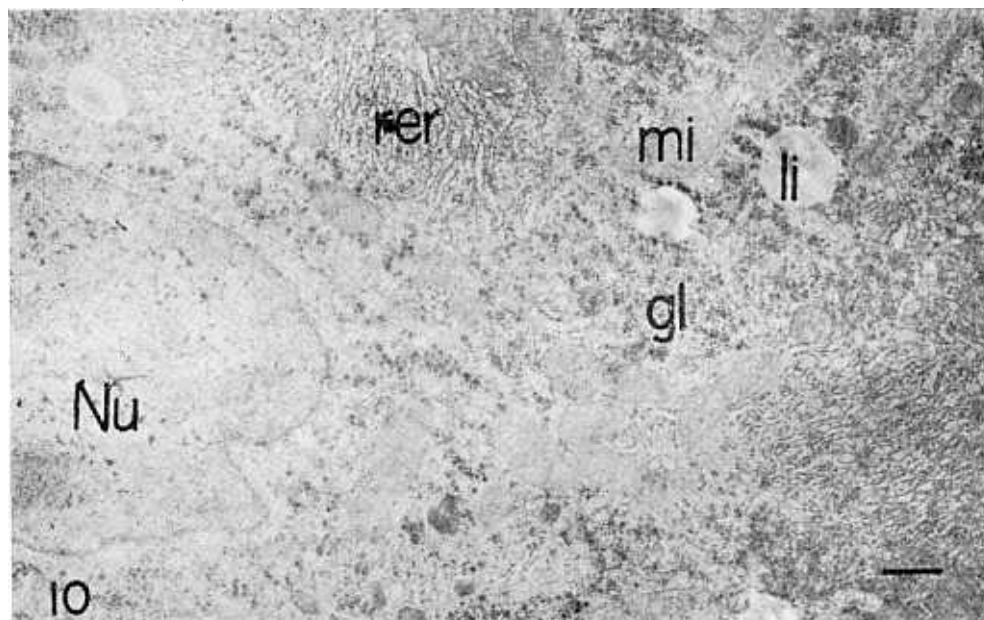


Fig. 10. Irregularity of the nuclear envelope, dilatation of rough endoplasmic reticulum, increased number of glycogen and lipid droplets were also observed by the treatment of Experimental strain of *Aspergillus* A-56. rer: rough endoplasmic reticulum, mi: mitochondria, gl: glycogen, li: lipid droplet, Nu: nucleus.