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Toxicity of mushroom samples in cell culture system

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The possible toxicity of fresh and dried mushroom samples was studied in cell culture system. The specimen were small, a piece of a basidiocarp or one basidiocarp of small size mushroom. A sterile extract prepaired from the sample was studied in U-cell culture. In the test system 0.2 ml of the extract was added in 2.5 ml of the cell culture medium. The toxic effect was seen in 1-2 days. The method makes possible to study the biological toxicity of small size samples like one small basidiocarp. Both toxic and non-toxic specimen were found within one species when studied in cell cultures. Some specimen (*Amanita muscaria*) were fed to mice also and studied in U-cell cultures by way of comparison.

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Cell culture and tissue culture methods have proved useful in toxicology both for detecting the toxic effect of substances and for studying the mechanism of the effect. A multitude of different cell and tissue culture systems are in use in laboratories all over the world (Rofe 1971, Worden 1974).

Some mushroom toxins are well known in their chemical structures and occurrence in different species, but information is lacking about the toxic substances of a great number of species found toxic only in some cases.

The purpose of this work was to study 1) whether the cell culture method is useful for the detection of toxic substances in mushrooms; 2) whether there are toxicity differences among samples from a single mushroom; 3) how toxicities found by cell culture method compare with the known toxicity of different mushrooms.

Material and methods

Preparation of mushroom material

The stipe was removed and a sector weighing 1-3 g was cut from the fresh pileus. Small caps were used whole, and the caps of 2 to 4 very small mushrooms were occasionally put together to give sufficient material. The sample sizes of the dried mushrooms were 200 to 1 000 mg. The fresh or dried sample material was cut into small pieces or ground in a mortar. Two ml of water was added and the sample was kept for two hours at +4° C; thereafter it was centrifuged at 3 200 r/min for 20 min. The clear supernatant was filtered through a Millipore filter (pore size Ø 0.22 µm) before being studied in U-cell cultures.

U-cell toxicity test

The U-cells (U-4, Orion, originating from human amnion) had been cultured as a continuous cell line in the laboratory. Tests were carried out in small plastic Petri dishes (2 cm Ø). For each test 2.5 ml of U-cell suspension mixed with Eagle's minimum essential medium containing 10% calf serum, 0.03% glutamine, 100 I.U./ml of penicillin, and 100 µg/ml of streptomycin was added to 0.1 ml of sterile sample of mushroom extract. The samples were checked after 24 and 48 hours. Often all the cells died within 24 hours. Samples which caused clear degeneration of the cells within 24 hours regarded as toxic.

Feeding experiment on mice

Amanita muscaria: Fourteen samples of *A. muscaria* were proved non-toxic to U-cells. The dried material serving as the source of these samples was fed to groups of ten mice at either 10% or 30% concentration in feed during a four-week period.

Cortinarius speciosissimus: The dried mushroom, source of the one sample of *C. speciosissimus* proving toxic to U-cells was fed to two mice at about 20% concentration in feed.

Results

Cell toxicity test

The results obtained with the U-cell toxicity test are shown in Tables 1 and 2.

Feeding experiment on mice

A. muscaria: During the first days of the experiment the mice fed with 30% of *A. muscaria* ate little and were in poor condition. At the end of the four-week experiment all the mice were well and no macroscopical or histological changes were found.

C. speciosissimus: The two mice fed with *C. speciosissimus* died on the sixth day of the experiment. There

was degeneration at tubulus cells in the kidneys.

Reference toxins

DL-muscarine chloride (Sigma) and gyromitrin (synthetic) were tested as reference toxins. They were found toxic to U-cells, under the conditions used, at concentrations of 0.1 mg/ml and 5 mg/ml, respectively.

Table 1. Toxicity of fresh mushroom samples to U-cells

Name	Number of samples	Toxic to U-cells	Non-toxic to U-cells
Amanita citrina S.F.Gray	4	2	2
A. vaginata (Fr.) Vitt.	1	1	
Armillariella mellea (Fr.) Karst.	11	0	11
Boletus edulis Fr.	1	1	
B. piperatus Fr.	2	2	
B. subtomentosus Fr.	1	1	
Cantharellus tubaeformis Fr.	7	0	7
Clitopilus prunulus (Fr.) Kumm.	2		2
Collybia acervata (Fr.) Kumm.	5	5	
C. butyracea (Fr.) Quél	1		1
C. maculata (Fr.) Quél	1		1
Coprinus atramentarius (Fr.) Fr.	2	2	
Cortinarius armillatus (Fr.) Fr.	2	1	1
C. bovinus Fr.	1		1
C. speciosissimus Kühn. & Romagn.	7	1	6
C. violaceus Fr.	4	4	
Dermocybe cinnamomea coll.	2		2
D. sanguineus (Fr.) Wünsche	1		1
Gomphidius glutinosus (Fr.) Fr.	4	3	1
G. maculatus Fr.	3	1	2
Gymnopilus penetrans (Fr.) Murr.	2	1	1
G. picreus (Fr.) Karst.	4		4
Hebeloma sp.	5	2	3
Hygrophoropsis aurantiaca (Fr.) Schroet.	6	6	0
Inocybe geophylla (Fr.) Kumm.	12	4	17
Kuehneromyces mutabilis (Fr.) Sing.& A.H.Smith	14	6	8
Lactarius deliciosus(Fr.) S.F. Gray	1		1
L. helvus (Fr.) Fr.	14	4	10
L. pubescens (Krombh.) Fr.	3		3
L. quietus (Fr.) Fr.	6	1	5
L. rufus (Fr.) Fr.	4		4
L. thejogalus (Fr.) S.F. Gray	8		8
L. torminosus (Fr.) S.F. Gray	1	1	
Leccinum scabrum (Fr.) S.F. Gray	2		2
Mycena laevigata (Lasch) Quél.	4		4
Naematoloma fasciculare (Fr.) Karst.	6	6	
N. sublateritium (Fr.) Karst.	7	7	
Oudemansiella platyphylla (Fr.) Mos.	1	1	
Paxillus atrotomentosus (Fr.) Fr.	4	4	
P. involutus (Fr.) Fr.	5	3	2
Pholiota astragalina (Fr.) Sing.	1	1	
Rhodophyllus centratus	1	1	
Rozites caperata (Fr.) Karst.	1		1
Russula consobrina (Fr.) Fr.	1		1
R. decolorans (Fr.) Fr.	1		1
R. emetica (Fr.) S.F. Gray	3		3
R. paludosa Britz.	5		5
R. vesca Fr.	2		2
Sarcodon imbricatus (Fr.) Karst.	1		1
Suillus grevillei (Klotzsch) Sing.	6	2	4
S. luteus (Fr.) S.F. Gray	2		2
Tricholoma album (Fr.) Kumm.	5	3	2
T. virgatum (Fr.) Kumm.	3		3

Table 2. Toxicity of dried mushroom samples to U-cells

Name	Number of samples	Toxic to U-cells	Non-toxic to U-cells
Albatrellus ovinus (Fr.) Kotl. & Pouz.	2	2	(slightly)
Amanita muscaria (Fr.) Hook.	1	1	(slightly)
Armillariella mellea (Fr.) Karst.	8	7	1
Boletus edulis Fr.	1		1
B. piperatus Fr.	1	1	
Cantharellus cibarius Fr.	1		1
C. tubaeformis Fr.	6	6	
Clitocybe nebularis (Fr.) Kumm.	1		1
Collybia maculata (Fr.) Quél.	2		2
Cortinarius spp.	27	7	20
Gomphidius glutinosus (Fr.) Fr.	1		1
Hydnum repandum Fr.	1		1
Hygrophoropsis aurantiaca (Fr.) Schroet.	2	2	
Lactarius helvus (Fr.) Fr.	1		1
L. mammosus (Fr.) Fr.	1		1
L. necator (Fr.) Karst.	1		1
L. rufus (Fr.) Fr.	1		1
L. trivialis (Fr.) Fr.	1		1
Lepista nuda (Fr.) Cooke	1		1
Lyophyllum connatum (Fr.) Sing.	1		1
Naematoloma sublateritium (Fr.) Karst.	1		1
Panellus serotinus (Fr.) Kühn.	1		1
Paxillus involutus (Fr.) Fr.	8	8	
Russula xerampelina (Secr.) Fr.	1		1
Sarcodon imbricatus (Fr.) Karst.	1		1
Stropharia aeruginosa (Fr.) Quél.	1		1
S. hornemannii (Fr.) Lund. & Nannf.	3	2	1
Suillus variegatus (Fr.) O. Kuntze	1		1
Tricholoma flavovirens (Fr.) Lund.	2	2	
Tylopilus felleus (Fr.) Karst.	1	1	

Discussion

The known mushroom toxins can be divided into the following groups: 1) cell toxins (amatoxins, phallotoxins, gyromitrin, orellanin); 2) nerve toxins (muscarine, muscimol, muscazon, iboten acid, bufotein, psilocybin, psilocin); 3) gastrointestine irritants (chemically unknown); and 4) toxins with a disulfarim-type effect when used together with alcohol (Gulden & Schumacher 1977).

The substances of the first group are either toxic to the cell nucleus or are protoplasma or membrane toxins. This type of toxin can usually be detected in cell culture systems. Theoretically, toxins of the second group should not be detectable in cell culture systems. The reference sample of muscarine, which as a parasympathetic stimulant falls in group 2, was nevertheless found to be toxic to the U-cell system used. Possibly some other effect of muscarine is expressed in its toxic effect on U-cells.

The raw extract used contains, in dissolved or suspended from, a great number of compounds. Quite possibly some of the compounds inhibit the growth of U-cells without being mushroom toxins in the ordinary sense. On the other hand, the majority of the raw extract samples tested were without toxic effect. No quantitative measurements were made of the toxicity in different samples. It was clear, however, that

whereas some samples had only slight toxic effect on U-cells, others caused the death of U-cells within a few hours.

A most surprising results is that samples both toxic and non-toxic to U-cells were found within one species. There are reports that the toxicity of mushrooms within one species may vary, but comrehensive studies on this subject are few (Tyler et al. 1966, Faulstich et al. 1973).

With mycotoxins it tends to be the rule that the toxin-producing capacity among different strains of a fungus species varies greatly from non-toxic to highly toxic.

Generally the results of the U-cell tests are in agreement with the known toxicity of the species in question if one assumes that some non-toxic strains and samples are to be found in species designated as toxic.

There are, however, some other striking differences when the results from the U-cell toxicity tests are considered against the common classification into edible and toxic mushrooms. Some samples of *Boletus edulis*, *Albatrellus ovinus* and *Armillariella mellea*, for example, are found toxic to U-cells. There are reported indications that *A. mellea* (Boespflug 1974) and *A. ovinus* (Prin 1974) would also be slightly toxic to humans. Faulstich & Cochet-Meilhac (1976) analyzed amatoxins from nine species, using two highly sensitive methods: radio-immune assay (RIA) and inhibition of RNA polymerase. Both these methods independently revealed very small amounts of amatoxins in *B. edulis* and *Cantharellus cibarius*. The amounts detected in *B. edulis* were 0.2 and 3.1 nanograms/g fresh tissue by RIA and by inhibition of RNA polymerase, respectively. The amount of amatoxins found by the same authors in *A. phalloides*, however, was 17.5 x 10^4 nanograms/g fresh tissue. The great quantitative difference in amatoxins that Faulstich and Cochet-Meilhac found in edible and toxic mushrooms is worth emphasizing. They go on to suggest that small amounts of amatoxins "representing the norm", may be essential for the normal development of Basidiomycetes, while the deadly toxic species represent an overproduction of these compounds.

Muscarin and gyromitrin, used as reference toxins in this study, were both found toxic to U-cells. Both amanitin and phallotoxin have been reported to inhibit RNA synthesis in several cell lines (Fiume 1967, Foá-Thomasi et al. 1976). Probably other mushroom toxins as well could be detected by cell culture methods.

Those mushroom samples of *A. muscaria* and *C. speciosissimus* that were tested by both animal experiment and U-cell method gave parallel results with respect to toxicity.

At the present stage of knowledge the cell culture method cannot be recommended as a sufficient basis for deciding whether a mushroom is toxic or not. But the high sensitivity and the possibility of testing small samples suggest the potential of the method for some studies on mushroom toxicity.

In particular, the present results raise interesting questions about the quantitative variation of toxins in mushrooms of the same species.

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