FAO/UNEP/USSR

International Training Course

«TRAINING ACTIVITIES ON FOOD CONTAMINATION CONTROL AND MONITORING WITH SPECIAL REFERENCE TO MYCOTOXINS»

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MYCOTOXINS — HISTORICAL BACKGROUND AND PRESENT-DAY NOTIONS

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Moscow, 1984

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Introduction

Mycotoxins -- secondary metabolites of microscopic fungi- are classed with the most dangerous contaminants of food products and fodders which occur in natural conditions. Mycotoxins are distinguished by high toxicity and many of them possess mutagenic, teratogenic, and carcinogenic properties. At present we know more than 250 species of various microscopic (mould) fungi which produce approximately 100 metabolites of varying toxicity giving rise to alimentary toxicoses of man and farm animals. The facts accumulated in recent decades lend themselves to a conclusion about universal spread both of mycotoxin producers and oftoxins proper. There are all grounds to believe that the number of isolated mycotoxins will continue to grow with further study of the role of toxin-forming microscopic fungi in alimentary toxicoses of man and animals of unestablished etiology.

The problem of mycotoxins, in recent years, has seen increased attention from scientists and also from statesmen and international organizations. This is due, firstly, to the fact that the real danger of mycotoxins for man's health has been demonstrated; secondly, to the abundance of mycotoxin producers in nature; and thirdly, to the impressive proportion of economic damage caused by mycotoxins. Considerable progress has been made at present in determining the chemical structure, properties, specific features of biogenesis, metabolism, and mechanism of action of many mycotoxins, in understanding their role in

the etiology and pathogenesis of a range of alimentary toxicoses.

In this lecture I will address myself to some stages in the development of mycotoxicology and to the analysis of present-day notions of mycotoxins which are particularly dangerous for man's health.

Some aspects of the history of mycotoxicology

Mycotoxicology as a science dates back more than 100 years With a degree of conditionality we may assume that it originated at the middle of the last century when it was for the first time demonstrated that ergots of rye - Claviceps purpurea - which cause a grave disease of man and animals (ergotism, "St.Anthony's fire"), belong to fungi. Important milestones in the history of mycotoxicology are studies of a disease caused by the consumption of grain affected with Fusarium graminearum ("drunken bread"); diseases of horses such as stachybotryotoxicosis and dendrodochiotoxicosis; mass disease of people with alimentary toxic aleukia; the disease of "yellow-stained" rice. Present-day mycotoxicology, however, has been taking shape only in two recent decades - since the discovery of aflatoxins which cause acute and chronic lesions of liver in many species of animals.

Ergotism

Ergotism has been known since ancient times. Mass outbreaks of this disease which in Western Europe were of a nature of toxidemics, have taken tens of thousands of lives. Thus, in Paris only in 1129 approximately 14,000 people died of ergotism.

This disease has a grave clinical picture; the main symptoms include acute pains and burning in the extremities, convulsions and contracture of extremities, the development of gangrene, the detachment of soft tissues, and not infrequently of extremities at places of joints. Very often the disease had a lethal outcome.

Ergotism sets in when sclerotiums of a fungus Claviceps purpurea enter an organism with food or fodder. It is precisely in the sclerotium stage that this species of fungus becomes highly toxic for man and animals. Claviceps purpurea affects many species of wild and cultivated gramineous plants: Agropyron, rye grass, foxtail, fescue, barley, oats, rye, wheat, etc. (a total exceeding 170 species).

The toxic element of ergots is a big group of alkaloids (Table 1).

When the cause of ergotism was understood (ingestion of cereals affected with ergots) and when highly effective methods of preventing the contamination of graincrops with ergots were elaborated (presowing chemical treatment of seed; cleaning of affected seed from claviceps purpurea sclerotiums, for instance by flotation; application of specific farming practices, etc.), this disease practically disappeared. However, under certain conditions local outbreaks of ergotism are still possible as is seen, for instance, from a recent case of ergotism, described by us, in a country of Central Africa.

Notweorthy are the specific extremal conditions which facilitated the development of the described case: there was a severe drought for three years, crop failure, and famine. The

I234

Table 1

Alkaloids of ergot

Alkaloids of lysergic and isolysergic acids	Clavine alkaloids
Ergine	Agroclavine
Erginin	Secaclavine (Chanoclavine-1)
Ergokornin	Chanoclavine-II
Ergokorninin	Costaclavine
Ergochristin	Cycloclavine
Ergochristinin	Dehydrolysergol-I
α-, β-Ergocryptines	Elymoclavine
α -, β -Ergocryptinines	Elymoclavine-O-β-D-fructoside
Ergometrine, Ergometrinin	Festuclavine
Ergosekalin, Ergosekalinin	Fumigaclavines A and B
Ergozin, Ergozinin	Isochanoclavine-I
Ergostin, Ergostinin	Isolysergol
Ergotamine, Ergotaminin	Isopennyclavine, Pennyclavine
Lysergic acid	Isosetoclavine
Isolysergic acid	Lysergen, Lysergin, Lysergol
Lysergid	Setoclavine, Norsetoclavine

I234

examination of the meagre stocks of food grain (mostly barley) in several localities which were struck by the natural calamity (a mountainous plateau approximately 3250 m above sea level, deprived of vegetation, water and cut off from the main territory of the country with a population exceeding 40,000) revealed a typical picture of total contamination with ergot solstiums. Approximately 150 cases of chronic poisoning with ergot (mainly the gangrenous form) were found in the foci of the disease. The patients demonstrated various degrees of gangrenous changes in the lower(at times upper) extremities; spontaneous amputation of lower extremities. The typical picture of contamination with ergots is supplemented by the observed murrain (mostly of sheep)which coincided in time with the commencement of the disease among humans. It should be emphasized that the contamination with ergot began with wild plants and only later on spread to cultivated graincrops.

One of the most important ways to erodicate the focus of contamination with ergot is a complete replacement of contaminated grain by healthy grain; destruction of the food and forage grain which was contaminated; detection of patients at an early stage of the disease and specialized medical treatment of the patients.

Stachybotryotoxicosis

Stachybotryotoxicosis is a grave disease of horses which occurs owing to feeding horses with coarse cellulose containing fodder contaminated with toxigenic strains of Stachybotrys alternan., fungus and which is characterized by the development of inflammatory changes and necroses of the digestive tract.

I234

The first cases of this disease were described in 1931 in the Ukraine, and only later on - in 1938 - it was understood that the disease is caused by the contamination of fodder (first of all, straw) with fungi of Stachybotrys alternans.

Along with the necrotic lesions in the gastro-intestinal tract, stachybotryotoxicosis is characterized by the development of a haemorrhagic syndrome, leukopenia, and agranulocytosis, acute cardiovascular insufficiency. In humans, contact with contaminated fodder or commercial raw material leads to the development of dermatitis or pneumoconioses.

A number of mycotoxins (Table 2) have been recently isolated from the culture of a toxigenic strain of Stachybotrys alternans (Table 2).

Table 2

Toxic metabolites isolated from the culture of Stachybotrys alternans

Toxic metabolites (mycotoxins)	Molecular formula	Molecular mass
Roridin E	$C_{29}H_{38}O_9$	514
Satratoxin C	-	484
Satratoxin F	-	542
Satratoxin G	$C_{28}H_{32}O_{11}$	544
Satratoxin H	$C_{29}H_{36}O_9$	528

Dendrodochiotoxicosis

Dendrodochiotoxicosis is a disease of horses which was for the first time recorded in 1937 in the Ukraine. It sets in when animals are given straw affected with microscopic fungi Dendrodochium toxicum. This mycotoxicosis features a rapid asymptomatic course and suddenly terminates with a lethal outcome. The autopsy reveals multiple haemorrhages in the organs of the thoracic cavity.

It has been recently demonstrated that this species of microscopic fungi can synthesize two mycotoxins of trichothecene group: verrucarin A and roridin A.

Alimentary toxic aleukia

Alimentary toxicaleukia (or septic angina) is a grave disease of man and animals associated with the ingestion of grain contaminated with Fusarium sporotrichiella. It has been shown that this species of microscopic fungi, as a rule, multiplies in the grain wintered under show with the production of toxins during snow thawing. Poisoning with the contaminated grain sets in, usually, after the passage of one to four weeks; it takes a grave course and not infrequently terminates in death. Poisoning caused by the ingestion of grain crops which have wintered in the field was for the first time noted in Kazakhstan and in Siberia in 1932-1933. A serious outbreak of this disease took place in northern Kazakhstan and the Orenburg region in 1944-1945. The initial symptoms are characterized by the inflammation of the mucus membrane of mouth and larynx; they are followed by a profound suppression of the function of the bone marrow and the development of leukopenia, anaemia, and throm-

I234

bocytopenia. In the gravest cases, against the background of a pronounced suppression of the immunoreactivity of the organism, the development of sepsis is observed. Recent studies have convincingly shown that microscopic fungi Fusarium sporotrichiella produce a range of highly toxic mycotoxins of the trichothecene group.

The discovery of aflatoxins

Early in 1960's a mass murrain of turkey-poults in a south-eastern part of the UK was noted. It was caused by an unknown disease characterized by the development of necroses of the liver and the proliferation of the biliary ducts. The search of the etiological factor of the so-called X-disease of turkey-poults has shown that the disease was not associated with any pathogenic microorganisms or viruses or with the presence of some known poison in the fodder. It was also noted that the epizootic was of a local nature (more than 80% of all cases were registered in a radius of about 100 miles around London) and caused by peanut flour added to the fodder of poultry. The peanut flour was imported from Brazil. The analysis of this flour revealed microscopic fungi Aspergillus flavus Link Fries.The use of the contaminated peanut flour as fodder produced the outbreak of the disease among pigs and calves. Some time later, in 1961, expressed carcinogenic properties of toxic peanut flour were also demonstrated.

It was shown in 1961 that partially cleaned methanol-chloroform extract of contaminated flour is capable of evoking a characteristic proliferation of the biliary ducts in one-day old ducklings. Toxic substances were isolated from the extract

and acquired a general name of aflatoxins. By thin layer chromatography aflatoxins were separated into fourcomponents, denoted by the colour of fluorescence in ultraviolet light as aflatoxins B_1, B_2 (blue fluorescence), G_1, and G_2 (green fluorescence). In 1962 the empirical formulas of aflatoxins were calculated and in 1965 their chemical structure was determined.

Present-day notions of mycotoxins

Many mycotoxins are potentially dangerous for man and farm animals. However, aflatoxins, ochratoxins, zearalenon and trichothecene mycotoxins are distinguished, among all mycotoxins, by their toxic properties and by their abundance in nature.

Aflatoxins

The family of aflatoxins includes at present , besides the four main representatives (aflatoxins, B_1, B_2, G_1, and G_2), more than 10 other compounds which are derivatives or metabolites of the main group: aflatoxins M_1, M_2, GM_1, B_{2a}, G_{2a}, P_1, Q_1, M_3, aflatoxicol, aflatoxicol H_1, sterigmatocystins, aspertoxin.

In natural conditions on plant subtrates, Aspergillus flavus and Aspergillus parasiticus synthesize, in the main, aflatoxins B_1, B_2, G_1, and G_2; as a matter of fact, aflatoxin B_1 is produced in the largest quantities while aflatoxin G_2 in the lowest amounts. It has been shown that these species of microscopic fungi may also sunthesize aflatoxins B_{2a}, M_1, aflatoxicol, sterigmatocystins, and aspertoxin.

I234

The absolute configuration of aflatoxin B_1 was determined

in 1967, and then in 1969 this structure was confirmed by a laboratory synthesis. The chemical name of aflatoxin B_1, in keeping with present-day nomenclature, is (6aR-cis)(2,3,6a, 9a)tetrahydro-4-methoxy-cyclopenta[c]furo[3', 2':4.5]furo [2,3-h][1]benzopyran-I, II-dione.

Aflatoxins are poorly soluble in water (10-20 μg/ml), insoluble in non-polar solvents but are easily soluble in solvents of medium polarity,such as chloroform,methanol,dimethylsulphoxide. They are relatively unstable in a chemically pure form and sensitive to air and light.One should note that aflatoxins are practically not destroyed in conventional cooking of contaminated food products. Aflatoxins are destroyed only by treatment with ammonia or sodium hypochlorite.

Chemical techniques of detection and identification of individual aflatoxins are founded upon their specific fluorescence in ultraviolet light (approximately 365 nm), different mobilities in thin layer chromatography, specific absorption spectra, and fluorescence. The most common methods of aflatoxin analysis include thin layer chromatography and high-performance liquid chromatography with fluorescent detectors.

Particularly noteworthy are aflatoxins M_1 and M_2 (metabolites of aflatoxin B_1) which are usually found in milk of animals (cows specifically) and hence are quite dangerous for the health of man and first of all, children.

Another metabolite of aflatoxin B_1 -- aflatoxicol- is also very important for toxicology. The reaction of the formation of aflatoxicol from aflatoxin B_1 is reversible, owing to which many authors consider aflatoxicol as a reserve form of

aflatoxin B_1 in an organism.

Aflatoxins P_1, Q_1, and H_1 are products of detoxication of aflatoxin B_1 in the membranes of endoplasmic reticulum of the liver with the participation of cytochromes P-450 of the hydroxylase system.

The family of aflatoxins includes also sterigmatocystins (sterigmatocystin, O-methylsterigmatocystin, 5-metoxy-sterigmatocystin, and dimethylsterigmatocystin) which are natural products of the activity of some strains of fungi belonging to the Aspergillus and Penicillium genera. Sterigmatocystins emit a dull brick-red fluorescence in UV light. Similar to aflatoxins, sterigmatocystin and dimethylsterigmatocystin have a pronounced carcinogenic activity.

Aflatoxins,without fail, are best studied among all known mycotoxins. Tremendous factual material accumulated by now helps us to class these compounds with the strongest hepatotoxic and hepatocarcinogenic substances, the danger of which for human health may be considered as proven. Aflatoxins are not only particularly dangerous but extremely abundant contaminats of food products. Aflatoxins have been found practically in all countries, all continents in different grain and oil-bearing products, in nuts, beans, coffee, and cocoa beans and in many other products of vegetable and animal origin.

Ochratoxins

Ochratoxins are a group of related metabolites of some species of Aspergillus ochraceus and Penicillium viridicatum. By their chemical structure they are isocoumarins linked by

1234

an amide bond to L-phenylalanine. Table 3 sums up some physicochemical properties of ochratoxins.

Table 3

Some physicochemical properties of ochratoxins

Ochratoxin	Molecular formula	Molecular mass	Melting point, °C	Absorption in UV light, E, nm
A	$C_{20}H_{18}ClNO_6$	403 (xylene) 85 -95 (benzene)	169	37800(213)
B	$C_{20}H_{19}NO_6$	369	221	37200(218)6900 (318)
α	$C_{11}H_9ClO_5$	256	229	3000(212)5600 (338)

As a rule,only ochratoxin A and more rarely ochratoxin B are found as natural contaminants of food products, other ochratoxins (ochratoxin C, methyl ester of ochratoxin A, methyl or ethyl esters of ochratoxin B) being isolated only in the laboratory from cultures of toxigenic strains of fungi. Acid or enzymatic hydrolysis of ochratoxin A gives rise to ochratoxin -α and L-phenylalanine. Ochratoxins belong to thermally stable compounds, and food products contaminated with them yield with difficulty to detoxication.

Ochratoxin A is a widely spread contaminant of food products and fodder found in many countries, including Denmark, France, Poland, Yugoslavia, Bulgaria, Sweden, Canada, USA, in

such products as wheat, rye, barley, oats (10-27500 µg/kg), maize (15-200 µg/kg, coffee beans (20-360 µg/kg), etc.

Unlike aflatoxins, ochratoxins are strongly tropic to kidneys and affect proximal tubules. Accumulating in tissues of farm animals (pigs primarily) consuming fodder contaminated with ochratoxins, they may reach food of man.

It is believed that ochratoxins are the etiological factor of nephropathy of pigs and poultry. The lesions of kidneys identical with nephropathy of pigs observed in natural conditions have been produced experimentally by feeding pigs with fodder containing a crystalline preparation of ochratoxin A.

Prolonged epidemiological observations give ground to suggest that ochratoxins are the cause of a human disease known as the Balkan endemic nephropathy.

Zearalenone

Zearalenone (lactone of phenolresorcylic acid) and its numerous derivatives (dihydrozearalenol, zearalenol, dimethylzearalen, O-methylzearalen, p-methylzearalen, zearalane, etc.) are products of metabolism of many species of the fungi belonging to the Fusarium genus. In natural conditions, zearalenone is frequently found as a contaminant of various fodder crops, primarily of maize (0-1-10.0 mg/kg), in many countries. Noteworthy are cases of finding high concentrations of zearalenone (0.3-53.0 mg/kg) in beer and some national sour beverages in Swaziland and Lesotho.

I234

Zearalenone has a well pronounced estrogenic action. Contamination of fodder with this mycotoxin is the etiological fac-

tor of estrogenic syndrome in farm animals, primarily in pigs (fertility reduction, vulvovaginites, abortions, sterility) and also in cows, poultry, and rabbits. It has been demonstrated that estrogenic syndrome in pigs, described in many countries, sets in when fodder is contaminated with zearalenone at concentrations ranging from 0.1 to 6.8 mg/kg.

Thus far there is no reliable information about unfavourable action of zearalenone on man. In this context we should pay attention to information about high concentrations or zearalenone in beer. We should bear in mind the possibility of accumulation of zearalenone and its derivatives in tissues of farm animals which are used for man's food. Finally, it should be emphasized that some derivatives of zearalenone have been recently used as growth stimulators of animals and are being widely produced commercially.

Trichothecenes

12, 13-epoxytrichothecenes are a group consisting of more than forty compounds of similar structure produced by many species of Fusarium fungi. The origin of the name of these mycotoxins is associated with mircoscopic fungi Trichothecium roseum from whose culture trichothecin was isolated, as metabolite.

Besides Fusarium, there are some other species which also produce trichothecenes: Myrothecium, Cephalosporium, Trichoderma, Calonectria, and Stachybotrys. It should be emphasized that under natural conditions only four representatives of this group have been determined as contaminants of food and fodder: T-2 toxin, nivalenol, deoxynivalenol, diacetoxycirpenol.

Some of the physical and chemical properties of trichothecene mycotoxins are summed up in Table 4.

By their chemical structure, trichothecenes may be divided into four groups. Group A covers T-2 toxin, HT-2 toxin, neosolaniol, T-2 tetraol, isoneosolaniol, scirpentiol, diacetoxyscirpenol, monoacetoxyscirpenol, trichodermin, trichodermol, etc.; group B: nivalenol, deoxynivalenol (vomitoxin), monoacetoxideoxynivalenol, fusarenon-X, nivalenol diacetate, trichothecalone, and trichothecin; group C: crotocin; group D: verrucarin A, verrucarin B, diacetylverrucarol, roridins A, D, E, H, satratoxins G and H, verrucarin J, etc. As contaminats of food products and fodder, trichothecenes of group A and B are particularly dangerous.

All trichothenoenes which belong to group A and B do not reveal absorption or fluorescence in the visible part of the spectrum which inhibits their detection in thin layer chromatography. At present, however, special techniques are elaborated which make it possible to identify trichothecenes by TLC; the TLC plates are treated with reagents which form stained or fluorescent derivatives with trichothecenes. Thus, when TLC plates are treated with concentrated sulphuric acid, trichothecenes of the group A form spots which emit blue fluorescence in the UV light. Trichothecenes may also be stained with p-anisaldehyde forming pinkish-violet compounds with trichothecenes of the group A and with yellow-brown compounds trichothecenes of the group B. 4(p-Nitrobenzyl)pyridine form blue compounds with trichothecenes of both groups. The derivatives of trichothecenes of groups A and B with nicotineamide-2-acetylpyridine emit

1234

Table 4

Some physicochemical properties of trichothecenes

Toxin	Molecular formula	Molecular mass	Melting	TLC Rf
T-2 toxin	$C_{24}H_{34}O_9$	466	150-151	0.40
HT-2 toxin	$C_{22}H_{32}O_8$	424		0.09
Neosolaniol	$C_{19}H_{26}O_8$	382	150-151	0.22
T-2 tetraol	$C_{15}H_{22}O_6$	298		0.32[x]
Diacetoxys-cirpenol	$C_{19}H_{26}O_7$	366	162-164	0.36
Monoaceto-xyscirpenol	$C_{17}H_{24}O_6$	324	173-173.5	0.21
Scirpentriol	$C_{15}H_{22}O_5$	282	189-191	0.35[+]
Nivalenol	$C_{15}H_{20}O_7$	312	322-223	0.42[+]
Fusarenon-X	$C_{17}H_{22}O_8$	354	91-92	0.77[+]
Nivalenol diacetate	$C_{19}H_{24}O_9$	396	135-136	0.90[+]
Deoxynivale-Nol (vomito-xin)	$C_{15}H_{20}O_9$	296	131-153	0.62[+]
Monoacetoxy-deoxynivale-nol	$C_{17}H_{22}O_7$	338	185-186	0.80[+]

Note: +) in a chloroform methanol system (5 : 1);
in all other cases - in a chloroform methanol system (98 : 2).

I234

blue fluorescence in ultraviolet light.

Acute intoxication with trichothecenes affects first of all the haemopoietic and immunocompetent organs. Characteristic is the development of a haemorrhagic syndrome, refusal from fodder, vomiting. Most of farm and laboratory animals are distinguished by high susceptibility to trichothecenes. LD_{50} of the T-2 toxin administered internally is 3.8-6.27 mg/kg.

Under natural conditions, trichothecenes have been found in high concentrations (up to 1800 µg/kg) in a number of cereals and in feed maize. There are all grounds to suppose that some alimentary toxicoses of farm animals are associated with the effect of trichothecene mycotoxins. It should be likewise remembered that the etiological factor of the mass disease of people with alimentary-toxic aleukia which occurred in 1944-45 is the effect of microscopic fungi of the Fusarium sporotrichiella species which, as it has been recently demonstrated, produce the T-2 toxin and other trichothecenes.

There are no doubts that new information on the abundance, toxicity, and the mechanism of action of trichothecenes will make it possible to evaluate the danger of this vast group of mycotoxins for man's health.

Patulin

Patulin is a carcinogenic lactone which under natural conditions is found in apples, pears, and other stone fruit and berries, as well as in the products of their processing—juices and pastes. The producer of patulin are many species of microscopic fungi of the Penicillium and Aspergillus genera

but the most frequent producer of patulin is Penicillium expansum.

By its chemical structure, patulin is a 2,4-dihydroxy-2H-pyran-3(6H), 6-acetate-3,4-lactone with a molecular formula of $C_7H_6O_4$. Its melting point is 111°; it is well soluble in water and organic solvents with the exception of petroleum ether; it has one absorption peak in ultraviolet light at 276 nm. Initially it was isolated as an antibiotic but owing to its pronounced toxic and carcinogenic properties, it did not find any application.

From the practical point of view it is important that patulin is found not only in the rotten part of fruit and vegetables but also in the normal partes. In tomatoes, for instance, it is spread evenly throughout the vegetable. Contamination of fruit, vegetables, and berries with patulin, in natural conditions, may reach a very high level. There are instances when patulin's concentration in a rotten apple was as high as 17.7 mg/apple and in the market-able specimens of apple juice 1.0 mg/l. There is a noteworthy instance of contamination with patulin of sea buckthorn berries at a concentration exceeding 50.0 mg/kg. Patulin producing fungi frequently affect bread and other bakery products but on this substrate patulin is quickly inactivated, as it is believed, owing to the substances which incorporate sulphydryl groups.

Patulin belongs to highly toxic substances: its LD_{50} for mice in case of intraperitoneal administration is close

to 8.0 mg/kg, and for rats 4.6 mg/kg. In experiments with mice

patulin demonstrated teratogenic properties.

There are no convincing data about the danger of patulin for man's health. Some authors, however, believe that the high frequency of cancer of the oesophagus in Normandy is associated with a high level of contamination of fruit, juices, and cider with patulin.

Yellow rice toxins

(citreoviridin, citrinin, luteoskyrin and cyclochlorotin)

By the middle of the 1950's, cases of grave alimentary toxicoses were recorded in Japan. They were denoted as "yellow rice disease". The decoding of the cause of this disease has indicated that the etiological factor was the contamination of rice with microscopic fungi of the Penicillum genus.

C i t r e o v i r i d i n is a yellow pigment with pronounced neutrotoxic properties (it causes paralysis and disturbances of the central nervous system, of the respiratory and the cardiovascular systems) and it is produced by Penicillium citreo-viride fungi. By its chemical structure, it is a carbocyclic polyene with a molecular formula $C_{23}H_{30}O_6 \cdot CH_3OH$; melting point is 107°-111°; absorption maxima are at 234, 286, 294, and 388 nm; it is well soluble in ethanol, ether, benzene, chloroform, and acetone; it is insoluble in water and n-hexane. The toxicological picture caused by citreoviridin is very close to the set of symptoms of cardiac beri-beri.

Citrinin is a metabolite of microscopic fungi Penicillium citrinum and also of some other species of Penicillium and Aspergillus. The empirical formula of citrinin is $C_{13}H_{14}O_5$;

molecular mass is 250; melting point is 175°; absorption maxima in ultraviolet light are at 222, 253, and 319 nm; it is soluble in most of organic solvents. Citrinin possesses an expressed nephrotoxic action: at concentrations ranging from 62.5 to 500 mg/kg of fodder it produced the enlargement of kidneys and degenerative changes of the epithelium of kidney tubules in chicken. It is believed that along with ochratoxin A, citrinin is the etiological factor of nephropathy of pigs. Under natural conditions it is found in wheat, barley, and rye. It is stressed that under natural conditions citrinin was not isolated from yellow rice.

The microflora of yellow rice includes Penicillium islandicum which produces more than 20 toxic compounds the most important among which are luteoskyrin, cyclochlorotin and erythroskyrin.

Luteoskyrin (8,8'-dihydroxyrugulozin). Molecular formula $C_{30}H_{22}O_{12}$; molecular mass is 574; melting point is 287°; it is insoluble in water, well soluble in most of organic solvents; absorption maxima in UV light are at 280.5; 436 and 357 nm. Acute and subacute intoxication with luteoskyrin leads to the development of necroses and the fatty degeneration of liver, chronic intoxication results in cirrhosis of the liver and hepatomas.

Cyclochlorotin is a chlorine-containing peptide with the molecular formula $C_{25}H_{36}N_5O_8Cl_2$(α-pyrrol-carboxyl-L- α - aminobutylil-L-seryl-β-aminophenylpropionyl-L-serineamide); molecular mass is 573; melting point is 251°; it is soluble in water and in 1-butanol. Cyclochlorotin is a highly toxic substance

(its LD_{50} for intravenous administration in mice is 0.3 mg/kg, and for subcutaneous administration 0.5 mg/kg). Kust as luteoskyrin, cyclochlorotin acts primarily upon the liver producing cirrhoses and fibroses; it also has carcinogenic activity.

Tremorgenic mycotoxins

Tremorhenic mycotoxins comprise a group of mycotoxins produced by microscopic fungi of the Penicillium and Aspergillus genera. The name derives from the effect caused by these mycotoxins - body tremor, spastic syndrome. By their chemical structure, tremorgens may be subdivided into four groups: 1) penitrems, 2) fumitremorgens-verruculogen, 3) paspalin and 4) triptoquivalin. Some data for tremorgens are listed in Tabla 5.

Tabla 5

Tremorgenic mycotoxins

Toxin	Molecular formula	Molecular mass	Absorption in UV light, ε,nm
Penitrem A	$C_{37}H_{44}NO_6Cl$	633	31500(233) 16200(295)
Penitrem B	$C_{37}H_{45}NO_5$	583	
Verruculogen	$C_{27}H_3N_3O_7$	511	
Paxillin	$C_{27}H_{39}NO_4$	435	
Fumitremorgen A	$C_{32}H_{41}N_3O_7$	579	
Fumitremorgen B	$C_{27}H_{33}N_3O_5$	479	
Triptoquivalin	$C_{29}H_{30}N_4O_7$	546	
Yantitrem A	$C_{37}H_{47}NO_6$	601	

Besides those included in the Table, tremorgenic mycotoxins include triptoquivalon ($C_{26}H_{24}N_4O_6$), penitrem C, yantitrems B and C ($C_{37}H_{47}NO_5$ and $C_{37}H_{47}NO_4$), territrems A and B ($C_{28}H_{30}O_9$ and $C_{29}H_{34}O_9$), roquefortin ($C_{22}H_{23}N_5O_2$), and paspalin.

Tremorgenic mycotoxins both in laboratory and farm animals produce tremor, ataxia, muscular stiffness, and convulsions. It is emphasized that tremorgens are a relatively frequente cause of alimentary toxicoses of farm animals: cattle, sheep, etc. Noteworthy is a recently described case of the poisoning of dogs with moulded cheese. The clinical picture of this case showed the predominance of the spastic syndrome (generalized muscular tremor, hyperkinesia, intermitting opisthotonus, clonic convulsions). The analysis of this cheese revealed the presence of penitrem A. There are all grounds to believe that some alimentary poisonings of people with undetermined etiology, in the clinical picture of which prevail the symptoms of the lesions of the central nervous system are associated with the ingestion of food contaminated with the producers of tremorgenic mycotoxins.

The tremorgenic effect on the laboratory animals (mice) is observed at very low concentrations of mycotoxins: 0.92 mg/kg for verrucologen administered intreperitoneally and 0.19 mg/kg for penitrem A. It has been demonstrated that the action upon subcortical centres plays an important part in the mechanism of the tremorgenic effect of these mycotoxins.

Rubratoxins

Two highly toxic metabolites - Rubratoxins A and B (Table

6) - have been isolated from cultures of Penicillium rubrum and Penicillium purpurogenum which frequently affect cereals and specifically maize.

Rubratoxins have a pronounced hepatotoxic and also mutagenic and teratogenic effect. Under natural conditions, these mycotoxins are, most likely, involved in the development of alimentary toxicoses of cattle, pigs, and poultry. Many laboratory animals are also susceptible to rubratoxins. LD_{50} for mice in intraperitoneal administration is 6.6 mg/kg for rubratoxin A, and 3.0 mg/kg for rubratoxin B; an for rats it is approximately 6,0 and 0.4 mg/kg, respectively. It is emphasized that the toxicity index of rubratoxins is considerably lower when it is administered internally (LD_{50} is 200-400 mg/kg for different species of animals).

Table 6

Some physicochemical properties of rubratoxins

Toxin	Molecular formula	Molecular mass	Melting point, °C	Absorption in UV, ε, nm
Rubratoxin A	$C_{26}H_{32}O_{11}$	520	210-214	4430 (252)
Rubratoxin B	$C_{26}H_{30}O_{11}$	518	168-170	9700 (251)

pR-toxin and other mycotoxins

Penicillium roqueforti

Recently information has been obtained indicating that some fo the microscopic fungi which are used in the food industry may also produce mycotoxins. It has been demonstrated

that Penicillium roqueforti which is widely used in the manufacturing of some sorts of cheese is a producer of highly toxic metabolites: PR-toxin, roquefortin, and isofumigaclavine. In laboratory conditions, the amount of synthesized PR-toxin may reach 30 mg/100 ml of the medium. By its structure, it is a terpenoid whose activity is associated with the aldehyde group; its molecular formula is $C_{17}H_{21}O_6$. Many farm animals and most of laboratory animals are susceptible to the toxic action of the PR-toxin. LD_{50} for rodents in the case of intraperitoneal administration ranges from 10 to 15 mg/kg. Acute poisoning with PR-toxin is characterized by the disturbance of locomotive coordination and paralyses. In mice the oedema of the lungs, brain, and kidneys has been observed as well as degenerative changes of the liver and kidneys.

Roquefortin ($C_{22}H_{23}N_5O_2$) and isofumigaclavine A are alkaloids by their chemical structure. They have been found in cheeses in a number of European and American countries: roquefortin at a concentration of 0.06 to 6.8 mg/kg and isofumigaclavine A up to 4.7 mg/kg of the product. LD_{50} for mice in the case of intraperitoneal administration is 15-20 mg/kg for Roquefortin and 340 mg/kg for isofumigaclavine.

Specific methods of analysis of these mycotoxins have been elaborated making it possible to monitor their content in food products.

Cytochalasines

Cytochalasines and codocytochalasines represent a group of highly toxic metabolites of very similar structure and origin produced by fungi.

Cytochalasine R ($C_{28}H_{33}NO_7$) was first isolated from rice infected with Aspergillus clavatus which was the cause of the death of a child as a result of alimentary toxicosis of unidentified etiology. LD_{50} for one-day old rats in the case of intraperitoneal administration is 0.98 mg/kg; for adult rats it is 2.6 mg/kg. Acute intoxication with cytochalasine E is distinguished by expressed oedema, degenerative changes of the liver, kidneys, spleen, and other organs. Intoxication with cytochalasine B produces radical disturbances in the permeability of capillaries.

Cytochalasines A, B, and G ($C_{29}H_{37}NO_5$) are metabolites of Helminthosporium dematicideum and cytochalasines C and D are produced by Metarrhizium anisopliae. Cytochalasine B has the highest toxicity in this group. Cytochalasine D was first isolated from food grain. The dogs which were administered cytochalasine in a dose of 1-2 mg/kg of body mass demonstrated symptoms of the injury of the central nervous system: disturbances of the locomotive activity, tremors.

Codacytochalasine-1 ($C_{30}H_{39}NO_5$) and codacytochalasine-2 ($C_{28}H_{27}NO_4$) have been found as metabolites of a highly toxic strain Phomopsis paspali which was isolated from millet. Codacytochalasine-1 in a dose of 2.0 mg/kg killed all test mice within 45 minutes after administration, and within 20 minutes if the dose was 5.0 mg/kg.

The discovery of cytochalasines as natural contaminants of food products classes them with mycotoxins which are of potential danger for man's health.

Alternaria Mycotoxins

Recent years are characterized by increasing attention to mycotoxins produced by fungi of the Alternaria genus. Quite frequently the representatives of the Alternaria genus infect cereals in field; toxigenic strains have been isolated from peanuts and tomatoes. Up to 90% of Alternaria samples isolated from different graincrops killed rats when added to the fodder; in other experiments 33% of the studied Alternaria strains were toxic for chicken and 60% killed mice.

By their chemical structure, the Alternaria mycotoxins belong to xanthones (alternariol and its methyl ester, altenuisol, altertenuol, altenuene, altenusine) or to anthraquinone pigments. Tenuasonic acid has the greatest toxicity in this group of mycotoxins. The producer of this acid are many species of Alternaria. Tenuasonic acid has been found in rice at a concentration of up to 2.6 mg/kg; its producers have been isolated from grain products, soya beans, sorghum, peanuts, tomatoes during ripening and in storage. The toxic action of these mycotoxins has been studied in mice, rats, dogs, guinea pigs, and monkeys. LD_{50} for mice is 81 mg/kg. Studies of the possible etiological role of tenuasonic acid in some halmatological diseases of man are believed to be promising.

Conclusion

In this lecture we have ventured to discuss in a very concise form some historical aspects of the problem of mycotoxins and present-day notions about a number of mycotoxins found as natural contaminants of food products, these myco-

toxins are distinguished by broad abundance and represent a potential danger to human health.

It should be emphasized that a large number of mycotoxins have been found in the study of microscopic fungi in laboratory. They have been studied in rather great detail and there are all grounds to believe that these mycotoxins or, at least, some of them, may be produced also in natural conditions and thereby be dangerous for human health. We are also witnesses of continuous isolation of new toxic metabolites of mould fungi which become new entries in the already long list of mycotoxins.

Modern mycotoxicology is a very multidimensional science and we are only at the initial stage of its development.

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Зак. 1234 ПИК ВИНИТИ