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

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MYCOTOXINS—BIOGENESIS AND MECHANISM OF ACTION

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MYCOTOXINS — BIOGENESIS AND MECHANISM OF ACTION

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INTRODUCTION

The study of principles governing the biosynthesis of mycotoxins by microscopic fungi, the determination of the sequence of reactions of biotransformation of mycotoxins in an animal organism, and the decoding of molecular and cellular mechanisms of their action are definitely the most important research trends in the area of mycotoxins. Considering that prevention of the formation of mycotoxins by producer fungi is the most effective measure of prophylaxis of alimentary mycotoxicoses in man and farm animals, one can fully appreciate the attention given by mycologists and biochemists to the peculiar features of mycotoxin biogenesis under various environmental conditions. It is likewise natural that all medical measures towards the removal or decreasing the extent of toxic action of mycotoxins upon man or animal should be founded upon thorough understanding of the metabolism of these foreign compounds and the mechanism of their action on the cellular and molecular levels.

This lecture takes up specifically these questions: biogenesis, metabolism and the mechanism of action of mycotoxins. It should be stressed that the mentioned aspects of the overall problem of mycotoxins are most intensively studied and this, in its turn, leads to a continuous supplementation, broadening and at times even alteration of our notions about the biochemistry of mycotoxins.

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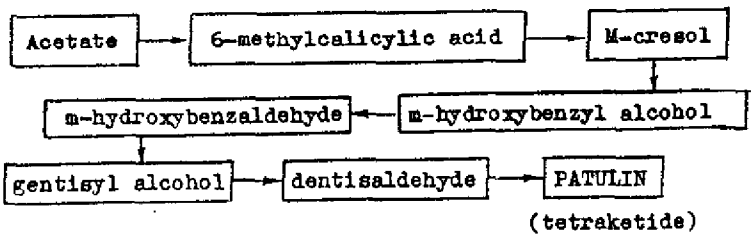
BIOGENESIS OF MYCOTOXINS

Mycotoxins are secondary metabolites of microscopic fungi and are formed from primary metabolites (acetate, malonate, mevalonate, amino acids) during enzymatic reactions of condensation, oxidation and reduction, alkylation and halogenation. Five basic pathways of mycotoxin biosynthesis are known — polyketide, terpenoid, through the cycle of tricarboxylic acids, through amino acids, and a mixed pathway which is a combination of two or more main pathways.

The polyketide pathway of mycotoxin biosynthesis

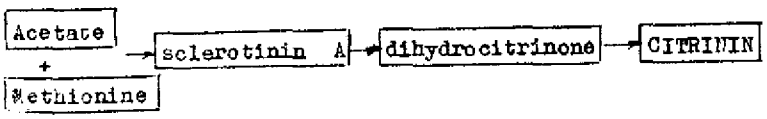
Most likely this pathway is the most important route of formation of mycotoxins, which is based on the reaction of linear condensation of acetyl-CoA with three or more molecules of malonyl CoA with accompanying decarboxylation but without the obligatory reduction of intermediate β-dicarbonyls. Let us consider this pathway of mycotoxin biosynthesis using several instances (in an increasing order of the number of included C_2 units).

The following sequence of metabolic transformations has been demonstrated for patulin biogenesis:

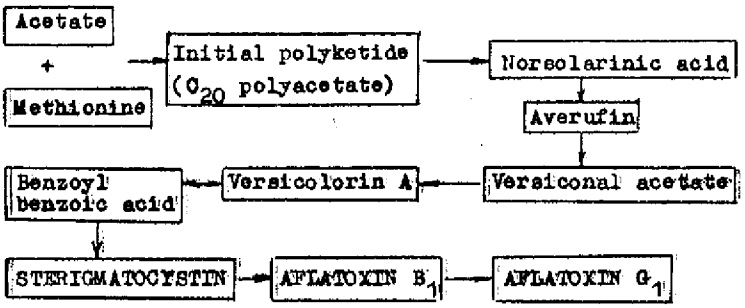


(tetraketide)

Pentaketides include citrinin and ochratoxin A:



Most likely, there is no need in giving a detailed description of the biosynthesis of individual representatives of polyketide mycotoxins. It would be enough merely to mention that octaketides are luteoskyrin and ergochromes; nonaketides -- zearalenon, citreoviridin and citochalazins; decaketides -- sterigmatocystins and aflatoxins. It goes without saying that the greatest study nas been made of the biogenesis of aflatoxins, the intermediate compounds of which have been isolated, identified and described:

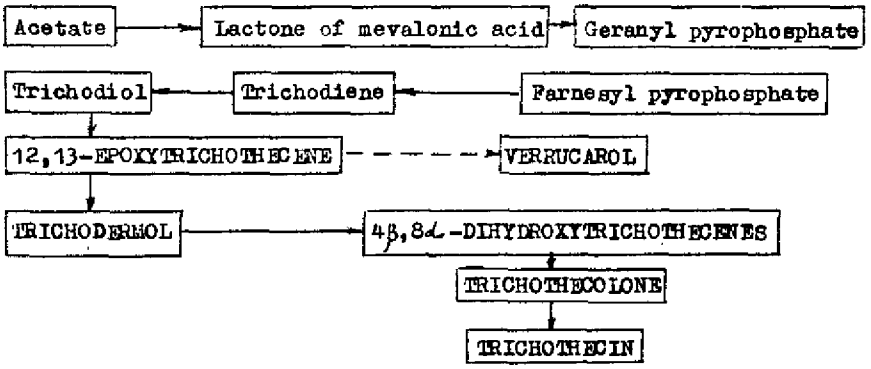


One of the most promising trends in aflatoxin research is the decoding of the ways of regulation of their biosynthesis with the idea of suppressing toxin formation. It has been demonstrated, for instance, that easily metabolizing hydrocarbons (galactose, fructose, ribose, maltose, etc.) intensify the aflatoxin synthesis by toxigenic strains of Aspergillus parasiticus. More than this, glucose or products of its metabolism are inducers (on the transcription level) of enzymes engaged in aflatoxin biosynthesis. It is noteworthy that cyclic AMP largely induces the aflatoxin biosynthesis. It is also important that the commencement of aflatoxin biosynthesis is preceded by a high rate of ATP and ADP formation, and that biosynthesis

proper is accompanied with a drop in the concentration of high-energy bonds. Also noteworthy is information about the inhibition of aflatoxin formation in the presence of benzoic acid and its derivatives, salts of molybdenum and vanadium. Thymol also has well pronounced antiaflatoxigenic activity.

Terpenoid pathway of mycotoxin biosynthesis

This pathway of biosynthesis is characteristic of a large group of trichothecene mycotoxins:

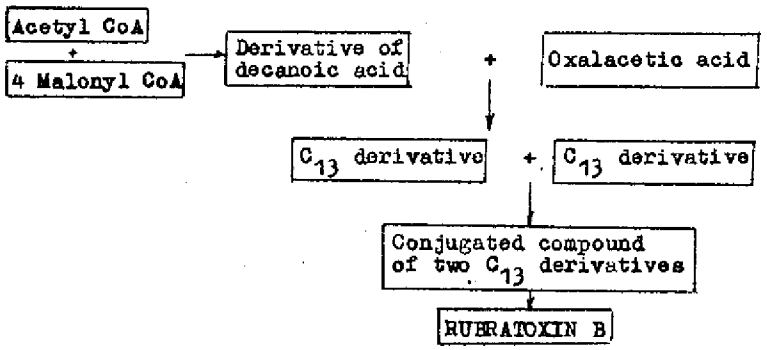


It is supposed that the biogenesis of the trichothecene skeleton is identical for all species of Trichothecium and Fusarium but the synthesis of individual specific trichothecene mycotoxins is predetermined by the distinctions in hydroxilation catalyzed by enzymatic systems which are genetically different in different species of fungi.

It should be also stressed that the biosynthesis of macrocyclic trichothecenes, such as verrucarines and roridines includes the stage of biogenetic combination of the isoprenoid residue with a polyketide.

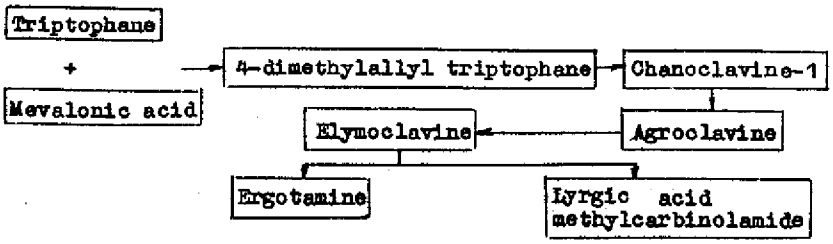
The pathways of biosynthesis of mycotoxins through the cycle of tricarboxylic acids

The pathway with the participation of metabolites belonging to the cycle of tricarboxylic acids is characteristic of the biosynthesis of rubratoxin B:



Pathway of biosynthesis of mycotoxins with the participation of amino acids

Amino acids are initial compounds in the biosynthesis of numerous mycotoxins. For instance, the pathway of biosynthesis of ergot alkaloids — ergotoxins, has been completely deciphered:



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The biosynthesis of a number of mycotoxins takes place through a combination of different ways of biogenesis, for instance, when amino acids and polyketides act as precursors

(erythroskyrin is synthesized with the participation of valine and a polyketide which is formed from acetate and malonate).

It should be stressed that the study of mycotoxin biogenesis is a most complex and painstaking affair based on the inclusion of labelled primary metabolites (acetate, amino acids, etc.) into the incubation medium of cultures of appropriate strains of microscopic fungi. Subsequent stages of research are the isolation of labelled secondary metabolites from the fungi cultures, identification and determination of their chemical structure, deciphering of the sequence of reactions of biotransformation. Notwithstanding all this, the deciphering of the ways of mycotoxin biosynthesis is theoretically and practically important because it is the main condition of the seeking out of ways of suppressing toxin formation by microscopic fungi.

BIOTRANSFORMATION OF MYCOTOXINS IN THE ORGANISM

Like many other xenobiotics, mycotoxins arrive in the organism mainly by intake in the gastrointestinal tract and via the portal vein reach the liver where they are detoxicated. The products of metabolism of xenobiotics are then excreted into bile and are withdrawn with the excrement or arrive in the kidneys and are withdrawn with urine. Biotransformation of mycotoxins proper, in an animal organism is a two-stage process embracing metabolization and conjugation. During the stage of metabolic transformations, mycotoxins are acted upon by appropriate enzymes and are oxidized, reducted, hydrolysed, etc. which leads to the development of functional groupings increasing the polarity of the molecules and being the sites of the next stage — con-

jugation, i.e. combination with such endogenous substances as glucuronic and sulphuric acids, amino acids, etc. During the process, the molecule of a mycotoxin or its metabolite becomes even more polar, its solubility in the lipid phase decreases and it is easily withdrawn from the organism. One should bear in mind that mycotoxin conjugation entails the blocking of functional groups, such as -COOH, -OH, etc.,the deactivation of the molecule, and thereby the reduction of its toxic properties. During the metabolic trnasformations new functional groups ordinarily develop in a mycotoxin molecule and this, as a rule, results in a loss of toxic properties. It is, however, most important to note that sometimes metabolization gives rise to compounds which possess, to the contrary, more pronounced toxic properties. This is known as metabolic activation.

Most of metabolic transformations of myctoxins in the organism take place in the liver with the engagement of enzymes of the endoplasmatic reticulum, so-called monooxygenases or oxidases with a mixed function. Biological oxidation, which is catalyzed by the enzymatic system of microsomes, incorporates a large number of reactions, but all of them may be reduced to a single common mechanism, viz. hydroxylation which calls for the availability of the reduced coenzyme $NADP \cdot H_2$ and O_2 : $RH + NADPH_2 + H^+ + O_2 \longrightarrow ROH + NADP^+ + H_2O$. The first stage of this reaction is the binding of a mycotoxin with oxidized cytochrome P-450; the second is the reduction of the developed complex in a $NADP \cdot H_2$-specific chain of electron transport; the third is the formation of a triple complex cytochrome P-450-mycotoxin-O_2; the fourth is the activation of O_2 in the complex by its reduction; the fifth is the decomposition of

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the complex into oxidized cytochrome P-450 and oxidized mycotoxin with the formation of a free hydroxyl.

Metabolism of aflatoxins

Numerous studies on different species of animals have shown that regardless of the ways of administering, aflatoxins are rapidly accumulated in the liver and that precisely the liver, in most cases, is the sole affected organ. The study of intracellular distribution of aflatoxin in liver tissue revealed that within 30 minutes after administration, approximately 30% of the initial amount of aflatoxin is concentrated in the nuclei of hepatocytes, and with the passage of 2 hours, more than 50% is associated with the microsomal fraction, i.e. localized in the endoplasmic reticulum where the main processes of biotransformation of xenobiotics take place. Let us take up the metabolic transformations of the main representatives of the family of aflatoxins — aflatoxins B_1, B_2 and G_1.

As follows from diagram 1, the hydroxylation of aflatoxin B_1 may produce aflatoxins M_1 (hydroxyl group in the 4th position) and aflatoxin G_1 (hydroxyl group in the 22nd position); the O-demethylation of aflatoxin B_1 gives aflatoxin P_1. It is important to bear in mind that all these main metabolites of aflatoxin B_1 are much less toxic, the toxicity levels bein 50 per cent, 1/18, and 1/20 of the initial figure, respectively.

Unlike all this, the same microsomal oxidases with a mixed function may perform the "activation" of aflatoxin B_1 with the development of metabolites with a more pronounced biological activity. Such metaoolites include aflatoxin B_{2a} and 2,3-epoxide of aflatoxin B_1. Aflatoxin B_{2a} is a hemiacetal of aflatoxin B_1 and easily forms Schiff's bases with amino acids, peptides, and

proteins, including enzymes. The action of aflatoxin B_{2a} is associated with the acute toxic effect of aflatoxin B_1. 2,3-Epoxide of aflatoxin B_1 is a hypothetical metabolite since up to this time it has not been isolated in a pure form and there is but indirect evidence of the possibility of its formation in the endoplasmic reticulum of liver cells.

The possibility of the formation of this type of compounds is indicated by a sharp decrease in the biological activity of aflatoxins B_2 and G_2 having no double bond in the terminal furan ring, compared with aflatoxins B_1, G_1, and M_1. 2,3-Epoxide of aflatoxin B_1 easily forms adducts with nucleic acids. The carcinogenic effect of aflatoxin B_1 is associated with the action of this metabolite.

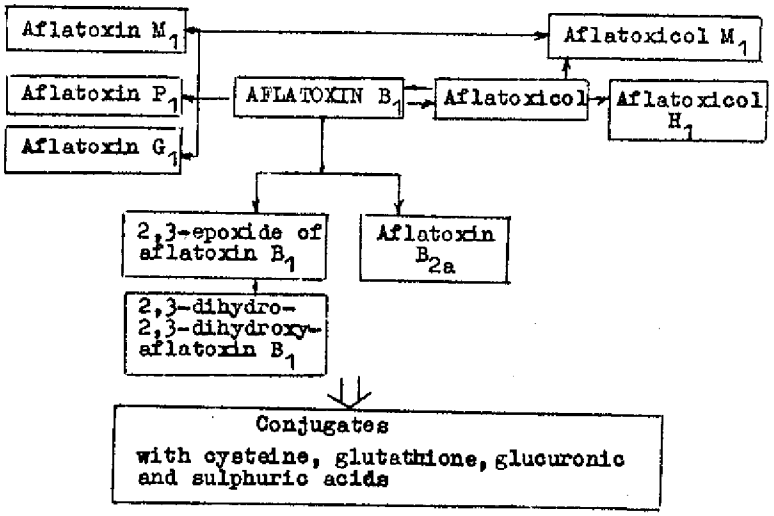

Diagram 1. Metabolism of aflatoxin B_1

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The biotransformation of aflatoxin B_1 may be carried out not only by the enzymatic systems of the endoplamic reticulum, but also by dehydrogenases in cytosol. In this case cyclopentenone is reduced to aflatoxicol; the process is catalyzed by NADP-dependent C-17 oxysteroid dehydrogenase. It should be specifically emphasized that this reaction is reversible which makes it possible to class aflatoxicol as a "reserve" form of aflatoxin B_1 in the organism.

Both aflatoxin B_1 by itself, and its metabolites may form conjugates with glutathione, cysteine, glucuronic or sulphuric acid with the participation of appropriate enzymatic systems.

The metabolic transformations of aflatoxin B_2 are more limited: this aflatoxin may be transformed into aflatoxins B_1, M_2, B_{2a}, and 2,3-dihydroaflatoxicol (Diagram 2).

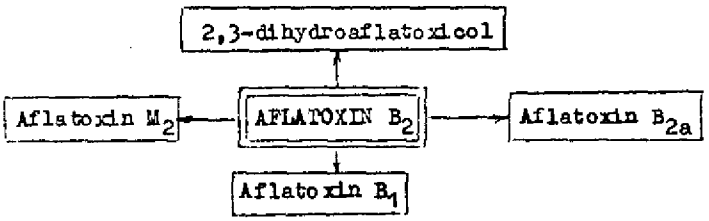

Diagram 2. Metabolism of aflatoxin B_2

The presence of slight carcinogenic properties in aflatoxin B_2 is explained by the possibility of its transformation into aflatoxin B_1. Therefore, the species of animals which metabolize aflatoxin B_2 into aflatoxin B_1 at a higher rate are more susceptible to the toxic and carcinogenic action of aflatoxin B_2.

Only three metabolic transformations are possible for aflatoxin G_1: the development of aflatoxins GM_1, G_{2a}, and 2,3-epoxide of aflatoxin G_1 (Diagram 3), whereas only one transformation (into aflatoxin GM_2) is known for aflatoxin G_2.

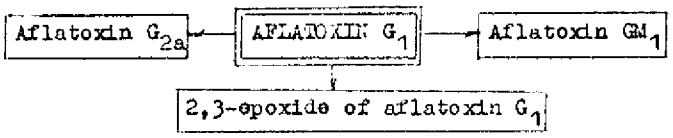

Diagram 3. Metabolism of aflatoxin G_1

Although up to now we have not elucidated any clear-cut correlation between the peculiarities of aflatoxin metabolism and the susceptibility of various animal species to them, there are all grounds to believe that, firstly, animals which actively metabolize aflatoxin B_1 into a hemiacetal (aflatoxin B_{2a}) are more susceptible to acute toxic lesions of the liver; secondly, animals which slowly metabolize aflatoxin B_1 with a possible development of such an active form as a 2,3-epoxide of aflatoxin B_1, are more subject to chronic carcinogenic action; thirdly, intense formation of aflatoxicol in the course of metabolism leads to aggravated acute toxic effects.

There are neither any doubts that the extent of toxicity of aflatoxin B_1 for different species of animals is greatly determined by the relationship of the rates of two enzymatic reactions: firstly, the reaction of epoxidation of aflatoxin B_1 with the development of 2,3-epoxide of aflatoxin B_1 and, secondly, the reaction of conjugation of 2,3-epoxide of aflatoxin B_1 with the formation of a glutathione-aflatoxin B_1 con-

jugate. Most likely, it is the high level of activity of the enzymatic system of detoxication (epoxydihydrase, glutathione epoxide transferase) that explains the resistance of some species of animals to the action of aflatoxin B_1.

Metabolism of ochratoxins

When ochratoxin A is administered into the organism of an animal, up to 95% of the compound is bound to blood proteins in the very first hours. After four hours, maximum amounts of labelled ochratoxin A are found in the kidneys, liver, and myocardium. More than 50% of the toxin in kidneys is localized in the cytosol and only 16% in the membranes of the endoplasmic reticulum.

It is believed that the main site of detoxication of ochratoxin A is the large intestine where with the participation of the microflora, enzymatic hydrolysis of a molecule of the toxin occurs with a splitting off of L-phenylalanine and the development of an inactive metabolite, ochratoxin α. It has been demonstrated quite recently that a certain proportion of ochratoxin A may be metabolized with the participation of microsomal oxidases with a mixed function to give two fluorescent derivatives: (4R)-4-hydroxyochratoxin A and (4S)-4-hydroxyochratoxin A (Diagram 4).

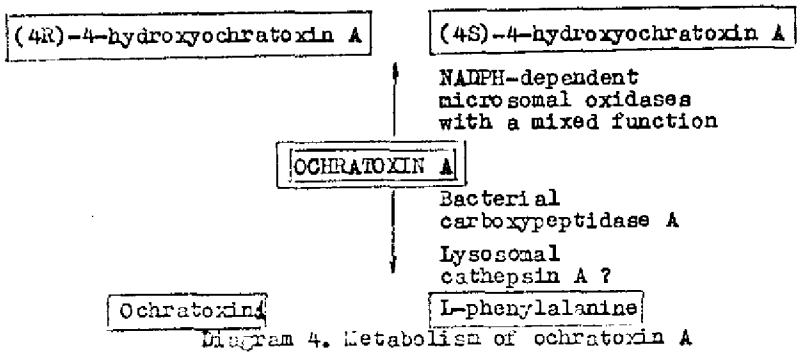

Diagram 4. Metabolism of ochratoxin A

Metabolism of zearalenon

When zearalenon is administered with feeds, it accumulates in maximum volumes in the adipose tissue, myometrium, ovaries, and liver. The biotransformation of zearalenon takes place both in cytozol (NADPH-dependent α-hydroxysteroid dehydrogenase), and in the endoplasmic reticulum (NADH-dependent α-hydroxysteroid dehydrogenase). It should be borne in mind that in the process of metabolism, α and β isomers of zearalenon, compounds which in terms of their estrogenic activity are from 4 to 5 times more active than the initial zearalenon, may develop during the metabolism. Both zearalenon and its metabolites may form conjugates with glucuronic and sulphuric acids. It should be likewise emphasized that in blood plasma of some farm animals (broiler chicks, for instance) zearalenon is preserved intact for a long time; it is therefore recommended to use this feature for the needs of diagnostics of alimentary mycotoxicosis.

Metabolism of trichothecene mycotoxins

When some trichothecenes (T-2 toxin, fusarenon-X) are administered internally, their maximum amounts are found in the liver within 30 minutes; somewhat lower concentrations are detected in the kidneys, bile, spleen, and intestines. After the passage of 3 hours, the maximum concentration of trichothecenes is found in the feces and urine. Within the first 96 hours more than 50% of the initial amount of trichothecenes is excreted with the feces, and from 12% to 17% with urine.

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It is believed that the metabolism of trichothecenes in the liver is implemented by the enzymes of the cholinesterase type which eliminate acetyl groupings in the C-4 position from the molecules of 12,13-epoxy trichothecenes, threrby trans-

forming T-2 toxin into NT-2 toxin, diacetoxy scirpenol into monoacetoxy scirpenol, fusarenon X into nivalenol. It should be, however, stressed that the processes of the biotransformation of trichothecenes are far from being fully studied and at present we cannot give a sufficiently detailed characteristic of the main pathways of metabolism of this group of mycotoxins.

Metabolism of patulin, citrinin, and some other mycotoxins

When given internally, only insignificant amounts of patulin are assimilated in an unaltered form in the gastrointestinal tract. Its maximum amounts have been found in erythrocytes, smaller amounts in the kidneys, spleen, lungs, and liver. It is believed that toxicosis, in the main, is caused by unknown metabolites of patulin. This mycotoxin is isolated primarily with feces and urine.

Citrinin is rapidly accumulated in the liver, kidneys and intestines within 30 minutes after administration. It disappears almost completely from blood plasma within 12 hours. Citrinin is isolated primarily with urine (more than 70% of the initial amount in 24 hours) and with feces (up to 10%). During first 6 hours, along with the unaltered citrinin, we may find in blood plasma two of its metabolites of unidentified structure. It is believed, however, that it is citrinin proper that has toxic action rather than its metabolites.

Rubratoxin B accumulates in the liver and kidneys within the first hour after administration, and approximately 80% of the toxin is localized in the cytosol of hepatocytes after the passage of 24 hours and about 10% in the endoplasmic reticulum. It has been demonstrated recently that the enzymatic systems of the endoplasmic reticulum which are induced by phenobarbital

are engaged in the development of less toxic metabolites of rubratoxin B, while the enzymes which are induced by 3-methylcholanthrene catalyze the formation of compounds with more pronounced toxic properties than the initial mycotoxin.

Luteoskyrin in subcutaneous administration to rats is rapidly accumulated in the liver where its concentration is maintained at a permanent level for 48 hours. The study of the intracellular distribution of labelled luteoskyrin in the liver of rats and mice has revealed that it is accumulated primarily in the mitochondria. Luteoskyrin is excreted primarily with feces and also with urine.

Two enzymatic systems are engaged in the metabolism of PR toxin which takes place in liver cells: the microsomal system which catalyzes the development of PR toxin alcohol; and the cytoplasmic system which catalyzes the formation of eremofortin in the presence of $NADPH_2$ C_3. Subsequently PR toxin alcohol may be turned into eremofortin C alcohol in the presence of $NADPH_2$ with the participation of the enzymes of cytosol. Eremofortin C may also turn into the same compound when acted upon by the enzymes of microsomes without the engagement of $NADPH_2$. It is supposed that during biotransformation of PR toxin into eremofortin C alcohol, the detoxication of the initial toxin occurs.

It follows from this information that only the metabolism of aflatoxins is sufficiently well studied. As for most of other mycotoxins, data about their biotransformation in the organism are very scanty or absent altogether. Nevertheless, it should be emphasized that further progress of mycotoxicology both in theoretical and practial aspects cannot be attained without deciphering the pathways of metabolism of individual mycotoxins.

THE MECHANISM OF ACTION OF MYCOTOXINS

To understand the ultimate biological effects of mycotoxins, one should not only learn the ways of metabolism but also possess detailed knowledge of cellular and molecular mechanisms of their action. In this part of the lecture we shall venture to sum up present-day notions about the mechanism of action of some of the most important mycotoxins.

Mechanism of action of aflatoxins

Numerous studies both *in vivo* on different animals and *in vitro* on various model systems have convincingly shown that the molecular mechanisms of aflatoxin effects are based on the interaction with DNA and thereby on the blocking of the DNA synthesis and of the DNA-dependent synthesis of RNA. It should be noted that the mechanism of aflatoxin action is intimately associated with the peculiarities of their metabolism. Here the processes of the so-called activation of aflatoxins in the cell acquire special importance. Thus, the decisive stages of the mechanism of action of aflatoxins are, firstly, the interaction of aflatoxin B_{2a} with amino acids and proteins and, secondly, the interaction of 2,3-epoxide of aflatoxin B_1 with nucleic acids.

The possibility of interaction of aflatoxin B_{2a} with amino acids makes one to presume the development, in an alkaline medium, of its phenolate ion whose aldehyde groups interact with amino groups of amino acids, peptides, and proteins to form Schiff's base (Diagram 5).

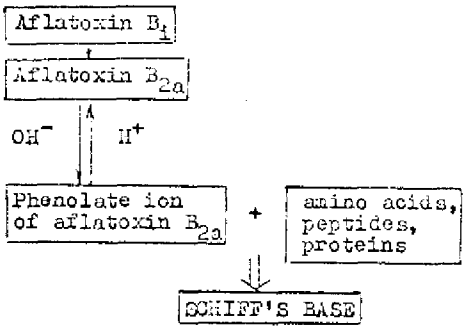


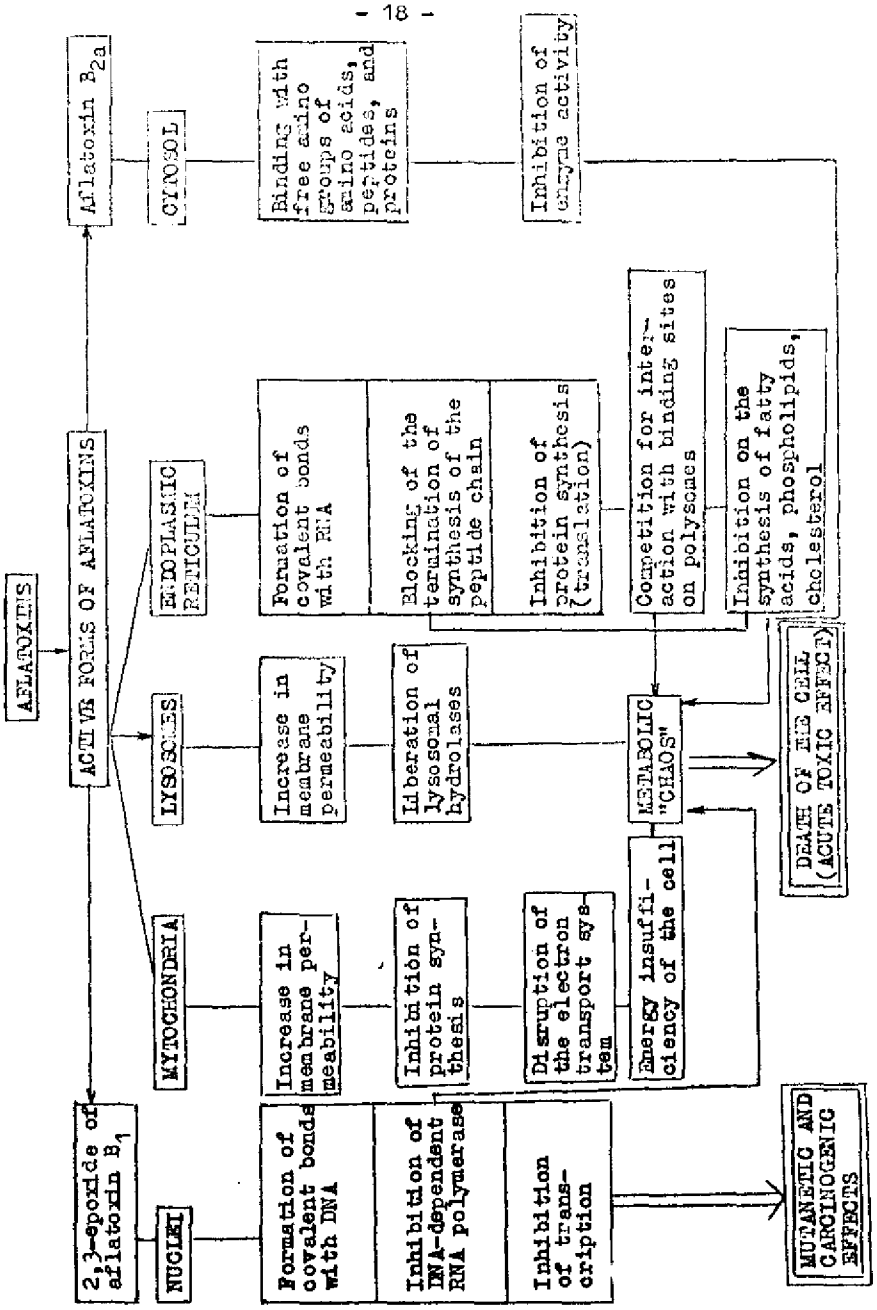
Diagram 6. Mechanism of interaction of aflatoxin B_{2a} with amino acids, peptides, or proteins

It seems that this particular mechanism underlies the inhibition of the activity of many enzymes in case of aflatoxicosis and that precisely aflatoxin B_{2a} is responsible to a greatest extent for the acute toxic effect of aflatoxin B_1.

Another active metabolite of aflatoxin B_1 — 2,3-epoxide of aflatoxin B_1 is particularly responsible for the mutagenic and carcinogenic effect of aflatoxin B_1.

The influence of aflatoxins upon the protein biosynthesis is not confined to their interaction with DNA and RNA. Recently it has been convincingly demonstrated that aflatoxins may block the termination of the peptide chain synthesis. This disrupts the motion of ribosomes along messenger RNA and their liberation which gives rise to so-called spiral polysomes which are excluded from the biosynthesis of protein.

While discussing the mechanism of aflatoxin effects, one should stress their damaging action upon various membrane structures of the cell, including mitochondria, lysosomes, endoplasmic reticulum, and nuclei (Diagram 6). It may be af-


AFLATOXINS
ACTIVE FORMS OF AFLATOXINS
Aflatoxin B_{2a}
2,3-epoxide of aflatoxin B_1
CYTOSOL
ENDOPLASMIC RETICULUM
LYSOSOMES
MYTOCHONDRIA
NUCLEI
Binding with free amino groups of amino acids, peptides, and proteins
Inhibition of enzyme activity
Formation of covalent bonds with RNA
Blocking of the termination of synthesis of the peptide chain
Inhibition of protein synthesis (translation)
Competition for interaction with binding sites on polysomes
Inhibition on the synthesis of fatty acids, phospholipids, cholesterol
Increase in membrane permeability
Liberation of lysosomal hydrolases
METABOLIC "CHAOS"
DEATH OF THE CELL (ACUTE TOXIC EFFECT)
Increase in membrane permeability
Inhibition of protein synthesis
Disruption of the electron transport system
Energy insufficiency of the cell
Formation of covalent bonds with DNA
Inhibition of DNA-dependent RNA polymerase
Inhibition of transcription
MUTANETHIC AND CARCINOGENIC EFFECTS

firmed with confidence that an important part is played by the disruption of the structure and function of cellular and subcellular membranes in the implementation of both the toxic and the carcinogenic effects of aflatoxins.

As it follows from the Diagram, aflatoxins or their metabolites in the nuclei are bound with DNA, inhibit the replication of DNA, DNA-dependent RNA polymerase, and the transcription proper; in mitochondria they increase the permeability of membranes, block the synthesis of mitochondrial DNA and proteins, disrupt the functioning of the electron transport system and thereby induce the energy insufficiency of the cell. Grave disturptions are also seen in the endoplasmic reticulium: first, it is the inhibition of the protein synthesis by interacting with messenger RNA and the blocking of the termination of the peptide chain synthesis on polysomes; second, it is a pronounced competition of aflatoxins for interaction with the binding sites on polysomes; third, it is the inhibition of the synthesis and the disruption of the control of the synthesis of fatty acids, phospholipids, and cholesterol. Aflatoxins in cytosol interact intensely with soluble proteins and inhibit enzymes. Aflatoxins act directly upon lysosomes, damage their membranes, and liberate lysosomal hydrolases.

Notwithstanding the abundance of experimental data, the biochemical mechanism of aflatoxin action cannot be taken as definitely determined. It is necessary, among other things, to clarify the contribution of each of the detected components to the final biological effects of aflatoxins.

As an intermediate product of aflatoxin biosynthesis, sterigmatocystin has a similar mechanism of action. It is believed that its metabolism involves the epoxidation of sterig-

matocystin and the formation of a highly reactive compound - 2,3-epoxide of sterigmatocystin. This particular metabolite entails alkylation of nucleic acids thereby inhibiting the protein synthesis. It is highly noteworthy that sterigmatocystin, similar to aflatoxins, induces the destabilization of the lysosomal membranes of the target organ -- liver, but to a smaller extent than it is done by aflatoxin B_1. This fact is in a definite correlation with the manifestation of the ultimate biological effect of sterigmatocystin, the mutagenic and carcinogenic action of which is less pronounced than in other representatives of the aflatoxin family.

The mechanism of action of ochratoxins

Ochratoxin A is a competitive inhibitor of phenylalanine -mRNA-synthetase and inhibits the synthesis of the polypeptide chain on the translation level. It is noteworthy that phenylalanine administered to mice in combination with LD_{100} of ochratoxin A completely prevents its toxic action. Phenylalanine also neutralizes the immunodepressive action of ochratoxin A. It is important to stress that chronic toxicosis induced by ochratoxin A may be likewise prevented by the administration of phenylalanine.

It has been also demonstrated in experiments on rats that ochratoxin A induces in kidneys the suppression of gluconeogenesis by inhibiting the activity of phosphoenolpyruvate carboxykinase. The specificity of the action of ochratoxin A is noteworthy as the toxin inhibited the activity of the enzyme only in the tissue of the kidneys but not in the liver.

The mechanism of action of zearalenon

Zearalenon and its metabolites have an expressed estro-

genic effect and stimulate the synthesis of DNA, RNA, and protein in target organs (uterus, mammary glands). It is supposed that the biological action of this group of mycotoxins is determined by their ability to competitively interact with specific estradiol-binding receptors in target cells.

The mechanism of action of trichothecene mycotoxins

Trichothecene mycotoxins are the strongest low molecular compounds which inhibit protein synthesis. Trichothecenes with more pronounced toxic properties, such as T-2 toxin, verrucarin A, nivalenol, diacetoxyscirpenol, scirpentriol, 15-acetoxyscirpendiol and fusarenon X, inhibit the process of initiation of translation, i.e. are included in the stage preceding the formation of a complex between the ribosome, messenger RNA, methionyl-mRNA including peptidyltransferase which is necessary for the first peptide bond. It has been demonstrated recently that fusarenon X and diacetoxyscirpenol inhibit initiation but on a stage when the first peptide bond is already established. It is important to stress that trichothecenes which inhibit the process of initiation of translation induce disaggregation of polysomes to monosomes.

Another group of trichothecenes, such as trichodermin, trichodermol, crotocol, trichothecolone, crotocin, trichothecin, verrucarol, and deoxynivalenol, inhibit the process of elongation or termination of the synthesis of the polypeptide chain, i.e. inhibit the binding of tRNA with ribosomes, and also the processes of translocation or prevent the liberation of polypeptides from ribosomes and the mRNA-ribosome complex. It should be stressed that all trichothecene mycotoxins which inhibit the protein synthesis also inhibit the activity of pep-

tidyltransferases by competing for the binding sites on ribosomes.

Information obtained recently indicates the capability of some trichothecene mycotoxins (nivalenol, diacetoxyscirpenol) to inhibit the synthesis of DNA along with the blocking of protein synthesis.

The mechanism of action of trichothecene mycotoxins is not confined only to the inhibition of the protein synthesis. An important component of the toxic action of some trichothecenes (T-2 toxin) is their damaging action upon the membranes of subcellular structures, specifically on the lysosomes of cells of haemopoietic and immunocompetent organs.

Mechanism of action of patulin, PR toxin, and other mycotoxins

Patulin inhibits the synthesis of RNA in the liver by blocking the initiation of transcription. It is important that is does not influence the stage of elongation. An essential component of the mechanism of toxic action of patulin is its active interaction with SH groups which leads to the suppression of the activity of most of thiol-dependent enzymes. Considering that patulin has an expressed antimicrobial action, the toxic manifestations of its effect may be also associated with the alteration of the bacterial flora of the intestines: the suppression of the Gram-positive flora and a sharp increase in the growth of Gram-negative flora. It should likewise be emphasized that toxic properties are characteristic of patulin proper, rather than of its metabolite which is formed under the effect of microsomal oxidases with a mixed function.

Unlike patulin, PR toxin which also inhibits the synthesis, of RNA suppresses the activity of the DNA-dependent RNA-poly-

merase and hampers both the initiation of transcription, and the subsequent elongation of the polynucleotide chain.

Citrinin is an inhibitor of the synthesis of sterols, it suppresses the activity of 3-oxy-3-methylglutaryl-CoA-reductase and glutamatedehydrogenase, which leads to the accumulation of substrates of the tricarboxylic acid cycle in the cell.

Luteoskyrin suppresses the synthesis of messenger RNA by interacting with DNA. A specific feature of the mechanism of action of this mycotoxin is a sharp disruption of the function of the mitochondria of liver cells which leads to the accumulation of triglycerides in them.

One of the important factors of the mechanism of toxic action of sporidesmin, a mycotoxin which causes the development of facial exzemas in ruminant animals, is its ability to generate superoxide radicals.

Rubratoxin B acts specifically on mitochondria by suppressing the activity of ATPase and blocking the electron transport chain. Another important aspect is the ability of rubratoxin B to inhibit the activity of microsomal oxidases with a mixed function which leads to a decrease in the concentration of microsomal protein and of cytochrome P-450, specifically.

The mechanism of action of tremorgenic mycotoxins (verruculogen, penitrem A) is based on the disruption of the process of liberation of neurotransmitters in synaptosomes (glutamate, aspartate, γ-aminobutyric acid). It is clear that an important factor of the development of tremor and convulsions under the action of fumitremorgens A and B is an increase in the level of serotonin and a decrease in concentration of γ-aminobutyric acid in the neurons of the brain.

CONCLUSION

Thus, we have discussed here three very important, both theoretically and practically, questions of modern mycotoxicology: biogenesis of mycotoxins in the cells of microscopic producer fungi; biotransformation of mycotoxins in the organism of animals; the mechanism of toxic action of mycotoxins.

At present, when there are no longer any doubts about the wide abundance and the real hazard of mycotoxins for man's health, the investigators are increasingly concentrating on the prevention of formation of mycotoxins microscopic fungi and on the weakening of their toxic action upon the organism. These problems can be solved only on the basis of detailed knowledge of the peculiarities of the biosynthesis, metabolism, and mechanism of action of mycotoxins.

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