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

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MYCOTOXINS: IMMUNITY, IMMUNOLOGIC METHODS OF STUDY

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Mycotoxins is the name given to the toxic metabolites of certain species of microscopic fungi. The danger of mycotoxins is connected with the fact that the microscopic fungi producing them are very widespread in nature and under certain conditions can affect fodder and food products.

At present, studies of the effect of mycotoxins on immunity acquire special significance. The practical value of this problem is determined by several factors;

- first, by contamination of food products and feed by mycotoxins and by high sensitivity of young animals, particularly chickens, ducklings, turkey poults, piglets, and calves, to the action of mycotoxins;

- second, by frequent use of these young animals for immunization and medical practice;

- third, by a prolonged (over 3 weeks) maintenance of the immune system defects against the background of normalization of physiological, biochemical, and cytologic parameters of vital activity after the cessation of mycotoxins administration to the animals.

There is no doubt that long-term defects of immunologic protection, caused by mycotoxins raise not only a medical but also a social problem. This problem is of particular significance to the developing countries because the weakening of immunologic control can be one of the main reasons for the increased spread of infectious, autoimmune, allergic, and oncologic diseases.

Consequently, one of the major requirements for an efficient control of the above pathological conditions lies in study of the immune system defects caused by mycotoxins. This task is particularly vital for two population groups: for children with their high sensitivity to mycotoxins and pathogens and also allergens of various origins, and for aged individuals much susceptible to malignant diseases and chronic conditions.

At present, the problem of prevention and control of toxic effects of mycotoxins, connected first of all with human health and environmental protection, is being resolved within the framework of international organizations.

Since the subject of this lecture involves such vast and independent field of science as immunology and mycotoxicology, it seems reasonable to discuss the following issues:

a) basic principles of the immune system functioning;

b) best studied mechanisms of the effect of mycotoxins on various elements of immunologic protection.

The notion (definition) of immunity and basic principles of the morpho-functional organization of the immune system

The modern notion of immunity refers to the way the organism protects itself against living bodies and substances possessing signs of genetically foreign information. The following can be included into the group of living bodies and substances bearing signs of foreign genome action: protozoa, microorganisms, viruses, proteins, modified cells and tissues including cancer cells. This definition is given to immunity on the basis of the

"Burnet axiom" which postulates that the central biological mechanism of immunity lies in the recognition of "its own" and "foreign". Thus, the basic function of immunological control consists in the protection of the stability of the internal medium of multicellular organisms against factors belonging to two basic groups.

The main group of these factors covers somatic mutations which lead to the formation of "foreign cells" differing from the organism's own cells by at least the minimal genetic sign, i.e. by one gene of histocompatibility.

The following facts prove that the main task of the immunologic control consists in the destruction of genetically foreign cells:

1) a more than 1000-fold increase of malignant tumors frequency among children with congenital defects of the immune system (mainly with thymus hypoplasia);

2) a sharp rise of lymphoma cases (36 times) and of reticulocellular sarcoma cases (350 times) among patients who have been subjected to a prolonged immunodepressive therapy in connection with kidney transplantation.

The second group of factors involves exogenous substances bearing signs of genetically heterologous information.

These facts allow us to group together all the substances which have signs of genetically heterologous information and which upon entering the organism induce specific immunologic reactions. The substances of this joint group are called antigens.

There are corpuscular (protozoa, bacteria) and soluble antigens.

It has been irrefutably proven that not only proteins and polypeptides, but also complex polysaccharides and lipopolysaccharides as well as high polymer forms of nucleic acids represent soluble antigens. All these compounds contain in their structure a specific trace of operation of heterologous genoms and possess the necessary molecular mass value.

Antigens, irrespective of the way they enter the organism, disturb the genetical stability of the organism's internal medium. As a result they are attacked by immune system cells recognising "its own" and "foreign".

The immune system is a set of highly specialized cells, tissues, and organs generalized throughout the body; the system exercises immunological control over the genetical stability of the organism's internal medium.

The following organs form the immune system in mammals; the thymus, spleen, lymph nodes, tonsils, Peyer's patches of small intestine, appendix, bone marrow and in the embryoliver. The organs of the immune system contain cells of several types: lymphocytes, plasma cells, macrophages, and granulocytes, all of which are called immunocompetent cells.

Lymphocytes are the main cells of the immune system. They are responsible for the principal manifestations of immunologic reactions;

- antibodies (immunoglobulins) synthesis;
- identification and elimination of heterologous cells (allotransplant rejection).

The above reactions objectively reflect the existence of two forms of immunity;

1) humoral which involves immunoglobulins generation;

2) cellular, which is due to the activity of sensitized lymphocytes.

The study of genetic defects of human immunity is of key significance in ascertaining the morpho-functional organization of the immune system. For example, the presence of congenital agammaglobinaemia (lack of resistance to viral and bacterial infections) together with a complete preservation of cellular forms of immunity (ability to reject transplants) proves undoubdtedly that there are two independent cellular populations in the immune system of mammals. Genetic defects cutting off both systems of cells can be considered as evidence of their common origin from one predecessor.

A thorough immunogenetic analysis has allowed a group of immunologists - WHO experts - to work out in 1977 a modern concept of cellular histogenesis of the human immune system.

The polypotent haemopoietic stem cell - HSC - is the ancestor of all cells of the immune system. This cell is localized in the embryonic liver, while in the postnatal period, in the bone marrow. The transformation of a haemopoietic stem cell into a lymphoid stem cell - LSC - is the major stage of the immune system development. The lymphoid stem cell is the common predecessor of two lymphoid cell systems: T and B lymphocytes.

T lymphocytes develop in thymus, the central organ of the immune system, under the influence of its epithelial cells (EC) and humoral factors (THF) - (thymosine and thymopoietin - which affect T lymphocytes outside thymus too.

As a result of the T cells development, at least three mature subpopulations of T lymphocytes develop: T helpers, T effectors and T suppressors. They circulate in blood and lymph

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and occupy peripheral organs. T cells account for 30-90% of all lymphocytes in human peripheral blood.

Cellular immunity reactions are ensured by T effectors. Under the influence of antigen stimulation T effectors propagate and lead to the accumulation of a clone of sensitized lymphocytes - killers - responsible for the elimination of genetically heterologous cells. Other subpopulations of T lymphocytes - helpers and suppressors - act as regulator cellulr of the immune response.

Thus, excessive activation of T suppressors inhibits the T effectors maturing, and this can be a reason for antitumor immunity defectiveness. In addition, it has been found that the process of B lymphocytes transformation into antibody producing cells requires T helpers assistance. T suppressors exert an inhibitory effect on this process.

In mammals, B cells mature in lymphoid tissue which can be considered as an analogue of bursa Fabricius in birds. The exact equivalent of bursa Fabricius has not yet been found in mammals; however, bone marrow and lymphoid tissue associated with the intestines (Peyer's patches and the appendix) may be regarded as such an equivalent.

From LSC, passing the stage of predecessors, immature bone marrow lymphocytes (IgMB) develop having IgM receptors on their surface. At later stages of maturation the lymphocytes acquire other surface receptors: IgG, IgA and IgD.

In addition to immunoglobulin receptors, B lymphocytes obtain other surface receptors in the course of maturation: for the C3 component of complement and for the Fc fragment of IgG.

The mature B lymphocytes penetrate the peripheral lymphoid organs. In human peripheral blood they account for 20-30% of all lymphocytes. Under the effect of antigen stimulation, the mature B lymphocytes differentiate into plasma cells producing antibodies of the IgM, IgG, IgA, IgE, and IgD classes.

Thus, the T and B populations of lymphocytes differ essentially in their histogenesis stages and in functions in the immune system.

The functional distinctions between T and B lymphocytes are naturally connected with the specificities of the biochemical structure of their plasmatic membranes bearing specific sets of surface receptors and antigens.

The plasmatic membranes of T lymphocytes, as compared with similar structures of B cells, are characterized by essential predominance (2-fold) of glycoproteids with N-acetylglucosamine and N-acetylgalactosamine, cholesterol (1.5 times), and cholesterol esters (almost 10-fold).

The plasmolemma of T lymphocytes, as compared with B cells, is characterized by predominance of the following receptors:

- susceptible to mitogens effect: phytohaemagglutinin (PHA) and concanavalin A (Con-A);
- binding Marek's disease virus;
- interacting with purified protein tuberculin (PPD).

In the cellular membranes of B lymphocytes there are much more receptors than in the plasmatic membrane of T cells;

- immunoglobulin receptors;
- receptors for the Fc fragments of immunoglobulins and for the C3 component of complement;
- receptors for pokeweed mitogen (PWM) binding.

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The plasmatic membranes of T and B lymphocytes differ not only in the above factors but also in many other markers. However, the above mentioned parameters are most important for the present subject.

When studying the immune response disturbance mechanisms, the above mentioned differences in receptors, specificity of antigens, and biochemical components of the membranes of T and B lymphocytes are used for the separation and identification of these cells in blood and peripheral organs.

It should be noted that a certain amount of lymphocytes (from 10 to 20%) do not possess T or B cell signs; these lymphocytes are called zero cells(zero lymphpcytes). They are considered to be the predecessors of cells which will complete their transformation into T or B lymphocytes.

In addition to T and B lymphocytes and plasma cells, macrophages play an important role in the immune response. Macrophages have a specific histogenesis: they originate not from LSC but from a haemopoietic stem cell and have a common predecessor with a granulocytic projection of haemopoiesis. Macrophages are also called A cells for their ability to adhere actively to glass.

Since phagocytosis and antigen catabolism provided by macrophages constitute an obligatory stage in antibodies synthesis induction, the scheme of antibodies genesis reactions proposed by the International of Immunologists Congress in 1977 takes into account cooperative interaction of T and B lymphocytes and macrophages.

Antigens with macrophages treated are identified by T lymphocytes-helpers. T helpers which have bound an antigen put B lymphocytes into antibodygenesis with the help of two signals.

The first signal, which is a specific one, represents a complex of IgM with the antigen; it is transported to a B lymphocyte by a marcophage.

The second signal, which is nonspecific, is of an unknown nature.

Thus macrophages and T helpers, by stimulating transformation of B lymphocytes into plasma cells, initiate antibody-genesis and intensify the biosynthesis of immunoglobulins.

Another subpopulation of lymphocytes - T suppressors - inhibits antibodygenesis by terminating the reproduction of the clone of plasma cells producing antibodies which results in the reduction of antibodies production.

Thus, T helpers which account for 60% of peripheral blood T cells and T suppressors covering 15% of T cells, represent the main regulators of antibodygenesis.

Antibodies synthesised by plasma cells, or immunoglobulins, are glycoproteids pertaining to the globulins fraction. Antibodies can specifically interact with antigens inducing their appearance. Due to this property, antibodies are one of the basic immunity factors directed specifically against the genetically heterologous substance which has induced their appearance.

As it was mentioned earlier, 5 classes of immunoglobulins are known: IgM, IgG, IgA, IgD, and IgE. They differ in the primary structure of polypeptide chains which form them. It is not our aim to consider the immunoglobulins structure in detail. The common principles governing their structure will be only discussed.

A molecule of any immunoglobulin contains three fragments. Two fragments specifically interact with an antigen by forming an active site of the antibody and imparting specific proper-

ties to it; they are marked as antigen - binding fragments - Fab. These fragments contain variable segments of polypeptide chains. The third immunoglobulin fragment is responsible for properties common to all antibodies of a given class: fixation on lymphoid, phagocytizing, and other cells, the binding of complement components. This fragment is marked as constant (Fc) and it contains constant segments of polypeptide chains.

Antibodies circulate in blood and other biological fluids. The basic volume of serum antibodies is represented by IgG - 70-80%, IgA - 10 -15%, and IgM - 5-10%. There is a rather small quantity of immunoglobulins of class D and especially of class E - about 0,01%;

The protective action of serum immunoglobulins against antigens inducing their appearance is manifested in their specific interaction with the antigens. The binding reaction of antibodies with antigens results in the formation of large aggregates which are called immune complexes. The formation of specific antigen-antibody complexes is aimed at the elimination of genetically heterologous agents and can manifest itself in several basic phenomena.

The precipitation phenomenon represents the enlargement (aggregation) and sedimentation of soluble antigens under the influence of antibodies.

The opsonization phenomenon is characterized by the strengthening of phagocytic activity of neutrophils and macrophages in relation to corpuscular and soluble antigens under the effect of specific immunoglobulins.

The agglutination phenomenon consists in the agglutination of suspended corpuscular (bacterial) antigens under the influence of specific antibodies.

Finally, the lysis phenomenon involves the dissolving of corpuscular antigens under the effect of specific antibodies in the presence of complement. As the complement system has been already mentioned several times, it is necessary to characterize it briefly.

The complement system consists of 9 protein components which in their turn may be divided into 11 independent proteins. Some complement components such as C1 and C3 are characterized by enzymatic activity - esterase and peptidase activity, respectively. Complement is found in serum of all mammals and its production does not represent a reaction to antigen injection. The activation of the complement components has the character of a cascade biochemical reaction and can be stimulated not only by immune complex but also without any help of antigens or antibodies. Active complement components, especially C3b C5+C6+C7, and C8+C9, induce a number of different effects; changes in blood coagulation, vascular permeability impairment, enhancement of phagocytosis, cytolysis. That is why the complement system occupies an intermediate position, it is referred both to the factors of nonspecific protection and to those participating in specific immunologic reactions.

After antigen-antibody reaction the Fc fragment of the immune complex interacts with the receptor zone of the C1 complement component, activising it. Activated C8 and C9- components of complement fix on the surface of the corpuscular antigen inducing the lysis of the cell.

Thus, the immune control function, manifested in cellular and humoral immunity reactions, requires a coordinated participation of several links of the immune system.

Immunity disturbances or immunodeficiency states develop mainly as a result of:

1) the blocade of the maturation of T and B lymphocytes

2) defects of regulating cells; T helpers and suppressors and A cells.

In addition to these factors, defects in the complement system result in the weakening of protective phenomena of immune complexes.

To evaluate functional ability of the immune system and to identify pathological changes in its work, multiple laboratory immunologic study methods are used. In the present lecture the immunologic methods used in studies of the effect of mycotoxins on the immunity reaction will be characterized.

Assessment of humoral immunity (B system)

1. The study of anibodygenesis. For this purpose animals are actively immunized by different antigens - ram erythrocytes, polysaccharide antigens of different origin, diphtherial and tetanic anatoxins, various vaccines. For a quantitative analysis of antibodies, the agglutination and precipitation phenomena are applied.

2. Biopsy of the lymph nodes, spleen, bone marrow. This procedure is performed for a histological identification of plasmatic (antibody-producing) cells and for the analysis of the structure of the lymphoid follicles.

3. Determination of the immunoglobulins level. Both total immunoglobulin concentration and the amounts of immunoglobulins of different classes are identified. In the first case the salting out of gross immunoglobulins by zinc sulphate followed by electrophoresis or immunoelectrophoresis is performed. The Man-

cini method of radial immunodiffusion is most frequently used for the determination of the amounts of immunoglobulins of different classes (IgM, IgG, IgA). The content of IgE is determined by the radioimmunologic method.

4. Determination of B lymphocytes. In view of the fact that B lymphocytes contain immunoglobulin receptors, the Kuns immunofluorescence method with the use of antiglobulin sera can be of help in finding the whole amount of B lymphocytes.

The presence of receptors to complement on B lymphocytes enables the determination of the number of complement rosettes (EAC); these lymphocytes form rosettes with ram erythrocytes bearing on its surface the antibody/complement complex.

5. Evaluation of the functional activity of B lymphocytes. This analysis becomes possible due to the fact that several mitogens, e.g. pokeweed mitogen (PWM) and antigen-bacterial lipopolysaccharides induce polyclonal stimulation of B lymphocytes. The lymphocytes subject to the effect of mitogen or antigen (originally or after sensitization) respond in blast transformation reaction. They increase in size and are characterized by a more intense synthesis of nucleic acids and proteins and by the appearance of mitoses. Quantitative evaluation of the blast-transformation test is done by the amount of ^{3}H-labelled thymidine in the lymphocytes.

Assessment of cellular immunity(T system)

1. Determination of T lymphocytes. The amount of T lymphocytes is determined by the method of spontaneous rosette formation. Formation of rosettes of lymphocytes with ram erythrocytes is also possible without preliminary immunization of ani-

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mals with ram erythrocytes. In such cases the number of rosette forming cells is proportional to that of T lymphocytes.

2. Assessment of the functional activity of T lymphocytes. To assess the functional ability of T cells, the blast transformation test is performed. In this case T lymphocyte specific mitogens - phytohaemagglutinins (PHA), concanavilin A (Con-A)- or antigens - tuberculin, purified protein tuberculin preparation (PPD), pathogens of parotiditis and Marek's disease - are selected. Quantitative monitoring of the T lymphocytes blast transformation test is performed by the level of traced nucleotides included in cells.

Besides the above mentioned tests used to evaluate the effect of mycotoxins on immunity reactions, the methods of determining the activity of the complement and marcophagal systems are also applied. The amount of complement is evaluated by haemolysis of 50% of antibody-sensitized erythrocytes, while the absorptive capacity of marcophages - by phagocytosis in opsonized erythrocytes and by the time of blood flow purification (clearance) from heterologous substances (colloid carbon or iron) Besides, investigators broadly use integrated indices of the immune system state manifested in the resistance changes in the animal organisms towards infectious pathogens under the effect of mycotoxins.

Such is the basic outline of the organization, functioning, and study of the immune system of the organism.

THE EFFECT OF MYCOTOXINS ON IMMUNITY

Study of the effect of mycotoxins on immunological processes has been started comparatively recently - at the end of the 60-ies - and conducted on a limited number of mycotoxins. The greatest number of studies has been performed in respect to aflatoxins, to be more precise, in respect to aflatoxin B_1 which biologically is the most active representative of four main aflatoxins (B_1, B_2, G_1 and G_2). Among other mycotoxins, ochratoxin A, rubratoxin A, T2 toxin, sterigmatocystin, stachybotritoxin, trichothecin and cytocholasin B have investigated.

It seems reasonable to consider the mycotoxin - induced impairment of immunologic reactions separately for cellular and humoral immunity because these two forms of immunity are due to the existence of two separate populations of major immunocompetent cells: T and B lymphocytes.

The effect of mycotoxins on humoral immunity

One of the first studies in this field was conducted by Kh.L. Galikeev et al. in 1968. The authors have demonstrated that a 3 days' subcutaneous injection of aflatoxin sharply reduced the titre of agglutinating antibodies in mice immunized with typhoid vaccine. In addition to this, more than a 50% decrease of plasmatic cells in the spleen and lymph nodes was observed. The above facts taken together permitted the authors to assume the aflatoxin-induced inhibition of the synthesis of antibodies.

Somewhat later Thaxton J.P. (1971, 1974) also observed the phenomenon of aflatoxin B_1-induced inhibition of antibodygenesis Any dose of aflatoxin from 0.625 mg/g to 10 mg/g given to broi-

lers resulted in a reliable reduction of the titre of haemagglutinins forming in the course of broilers immunization with ram erythrocytes. The degree of antibodies titre reduction depended on aflatoxin dose and feeding duration.

In the above mentioned works as well as in a study carried out by Pier A.C. et al. (1972) it was also found that the central organs of the immune system of turkey poults and chicken - the thymus and bursa Fabricius - lost weight under aflatoxin B_1 effect. The loss of weight of the thymus was more than 60% and that of bursa Fabricius - about 25%.

Other mycotoxins induce various effects on the humoral immune response (Richard J. et al., 1975, 1978). Thus, under the influence of 2-4 mg/kg of trichothecin and stachybotritoxin injected intraperitoneally in mice before ram erythrocytes immunization, the titre of haemagglutinins decreased. At the same time ochratoxin fed to quinea pigs in a dose of 0.45 mg/day during 4 weeks caused no inhibition of the immune response against antigens of brucellosis pathogens.

Thus, on the one hand, mycotoxins exert an immunodepressive effect on the humoral immune response, however, on the other hand, the influence of mycotoxins on immunity reactions turned out to be more complex than a simple inhibition of antibodygenesis.

The specific features of the effect of mycotoxins on the immune system manifested themselves in the following. In a series of studies carried out by Pier A.C. et al. (1970, 1972), the action of aflatoxin on the resistance of turkey poults and chicken immunized against a virulent strain of the fowl cholera pathogens Pasteurella multocida has been investigated. Aflatoxin was given to poultry with food in a dose of 0.25-0.5 mg/kg

in the course of 3 weeks.

It was found that aflatoxin caused similar disturbances of resistance to cholera pathogens both in turkey poults sensitive to the toxin and in chicken which were relatively resistant to it.

Impairment of resistance to P. multocida in turkey poults and chicken manifested itself in high death rate which reached 20-67% within 3-10 days after the challenging injection of the bacteria. At the same time injection of a challenging dose to aflatoxin-free immunized animals was not characterized by lethal outcomes.

The weakening of poultry resistance to cholera pathogens was accompanied with a manifested decrease of the general blood protein level and by almost a 50% reduction of the contents of albumins and α and β globulins. However, the level of γ globulins and, what is particularly important, the titre of agglutinins remained unchanged. Thus, the immunity impairment in turkey poults and chicken in respect to P. multocida was accompanied with the development of hypo- and disproteinaemia; however, in this case the process of antibodygenesis was not disturbed.

In further investigations it was found that aflatoxin admixture to poultry food (for turkey poults and chicken) in a concentration of 0.25-0.5 mg/kg reduced their resistance not only to cholera pathogens but also to other infectious pathogens, such as Salmonelle spp. (Smith C.A., 1969; Boonchuvit B., 1975), yeast flora- Candida albicans (Hamilton P.B. et al., 1971), Newcastle disease viruses (Pier A.C., 1973), and also to Marek's disease viruses and deep mycoses caused by coccidia Eimeria tenella (Edds G.T et al., 1973). Pier A.C. and

Heddleston K.L. (1970) established that the effect of aflatoxin on immunity is reversible.

The addition of aflatoxin to poultry food simultaneously with immunization or after it leads to the reduction of the resistance of turkey poults and chicken to cholera pathogens. When poultry immunization was carried out after termination of aflatoxin feeding, no impairment of resistance to cholera pathogens was developed.

On the basis of the results of these experiments the authors suggest that afletoxin B_1 does not simply weaken poultry immunity but impairs the very process of the immune response induction. The impairment requires aflatoxin presence in the organism in the period of its antigenic stimulation.

Out of other mycotoxins, T2 toxin fed to chicken in a dose of 16 μg/g as food admixture inhibited resistance of chicken to various Salmonella species inducing chicken paratyphoid. Rubratoxin, however, did not impair the resistance of turkey poults to the fowl cholera pathogens (Richard J. et al., 1978).

Ambiguous antibodygenesis changes under the effect of mycotoxins against the background of a manifested weakening of poultry resistance to cholera pathogens represented a sharp contrast with the earlier described facts of antibodygenesis inhibition in mice and chicken.

To make a more careful analysis, a group of research headed by Pier A.C. (1972) carried out a new experiment.

The basic group of turkey poults, like it was done earlier, received the same doses of aflatoxin with food and was immunized against P. multocida. Three other groups of poultry received additionaly:

1) normal mature turkey plasma;

2) immune plasma against cholera pathogens;

3) immune cells (mixture composed of peritoneal macrophages - 1/3 and leukocytes - 2/3).

The introduction of the challenging dose of cholera pathogens to poultry has confirmed a good reproducibility of the lowering of the resistance of turkey poults receiving aflatoxin. The death-rate in the group constituted 26%.

Immune plasma introduction to poultry up to resolution has reliably restored their tolerance which was confirmed by the absence of lethal outcomes.

Intact birds plasma has also demonstrated good protection properties while the mixture of immune cells (macrophages and leukocytes) has demonstrated a rather weak protective action.

The determination of agglutinating antibodies in serum fractions separated by ultracentrifuging in sucrose density gradient has shown that the maximum content of agglutinins was in the 9S, 12S, and 19S fractions; however, no great difference between aflatoxin recipients and control animals has been observed.

The cited data attract our attention to the fact that humoral factors of normal plasma participating in the development of infection immunity have pronounced protective properties and restore the impaired resistance.

These humoral factors localized in α- and β globulin serum fractions most susceptible to aflatoxin damage include the complement system components.

The nature of the activity changes of complement and main protein fractions of serum under the influence of aflatoxin and immunization has been elucidated in experiments by Thurston J.

and his colleagues (1972, 1974) performed on guinea pigs.

The animals were immunized against brucellosis pathogens- Brucella abortus - and received daily 0.05 mg of aflatoxin B_1 during 3 weeks.

A week later a noticable reduction (exceeding 30%) of the complement titre and a simultaneous reliable decrease of the content of the α_2 and β globulin fractions were observed. After two weeks the changes of these parameters became even more pronounced and were accompanied with a reliable reduction of the amount of the α_1 globulin fraction.

The content of albumins and γ globulins during this period was practically unchanged.

The investigators explain a severe inhibition of the complement (more than two-fold by the end of the second week) by a typical picture of liver damage by aflatoxin, the former being responsible for the synthesis of major volumes of α and β globulins.

Three weeks after aflatoxin introduction, a more than 50% decline of the levels of α_1 , α_2 , and β globulins and a noticeable growth of γ globulin content and complement activity were observed. However, despite an increase of γ globulins level, the titre of agglutinins against B. abortus was practically the same.

Thus, aflatoxin B_1 induces a manifested reliable decrease of the content of the α and β globulin serum fractions in poultry (turkey poults and chicken) and in mamals(guinea pigs). This fact is accompanied with a typical picture of aflatoxin-induced hystopathological damage of the liver and with the absence of change in antibodygenesis.

At the same time a considerable inhibition of the comple-

ment system activity was found in guinea pigs receiving aflatoxin.

In similar experiments performed with rubratoxin (6 mg/day) and sterigmatocystin (4.2 mg/day) fed to guinea pigs during 3 weeks, a decreased activity of complement was observed, mainly due to a diminished content of the C4 and C9 components (Richard J. et al., 1978). The addition of 0.45 mg/day of ochratoxin and T2 toxin and of 4 mg/day of rubratoxin to the food of guinea pigs during 3 weeks did not induce inhibition of the complement activity.

Besides the above mentioned facts of the effect of mycotoxins on humoral immunity reactions, Richard J. et al. (1975, 1978) report phagocytosis changes induced by mycotoxins. Thus, aflatoxin introduced into chicken diet in a dose of 0.625-5.0 μg/g during 3 weeks inhibited the phagocytosis of colloid carbon introduced intravenously in poultry. Inhibited phagocytosis by alveolar macrophages of rabbits receiving aflatoxin in a dose of 0.03-0.09 mg/day during 2 weeks was also noted. Another mycotoxin, cytocholasin B, in a dose of 0.5- 5.0 μg inhibited Staphylococcus aureus phagocytosis by rabbit alveolar macrophages and human basophils.

The investigators assume that inhibition of the complement activity considerably facilitating all phagocytosis stages can be a possible cause of phagocytosis impairment.

In addition to the above mentioned facts of the effect of mycotoxins on humoral immunity in vivo, some in vitro data on the effect of aflatoxin on immunocompetent cells have been obtained rather recently.

Thus, Paul P.S. et al. (1977) have found that aflatoxin B_1

inhibit the stimulation of peripheral lymphocytes induced by pokeweed mitogen (PWM).

The degree of lymphocyte stimulation was assessed by the intensity of tritium-labelled thymidine absorption by the culture of lymphocytes isolated from bovine blood.

The addition of aflatoxin in a 10-20 mg/ml concentration to the culture of peripheral lymphocytes has induced more than a two-fold inhibition of ^{3}H thymidine absorption. At the same time the viability of the cells remained practically unchanged.

We pointed out earlier that membrane receptors for pokeweed mitogen are typical both of T and of B cells but mainly of B lymphocytes. Consequently, the inhibition of the differentiation of B lymphocytes indicates the depression of the humoral component of the immune system in mammals.

Having discussed published experimental data on mycotoxin effect on humoral immunity in vivo and in vitro, it seems reasonable to study the effect of mycotoxins on cellular immunity reactions.

The effect of mycotoxins on cellular immunity

As it was mentioned earlier, the reduction of weight of the central organs of the immune system - the thymus and bursa Fabricius - when birds are fed with aflatoxin, is not the same in different animals. The thymus involution in turkey poults is manifested much stronger and constitutes more than 60% of the organ's mass.

Savol H. et al. found in 1970 that aflatoxin in vitro inhibits the transformation of human peripheral lymphocytes induced by a non-specific mitogen - phytohaemagglutinin (PHA) and

by specific antigens: PPD (purified protein - free tuberculin) and parotiditis pathogen.

A concentration of 5 mg/ml of aflatoxin caused a 54% inhibition of labelled thymidine absorption by the culture of lymphocytes stimulated by phytohaemagglutinin. A concentration of 50 mg/ml of aflatoxin induced a 62% inhibition of labelled thymidine absorption by the culture of lymphocytes stimulated by specific antigens - PPD and parotiditis pathogen - in patients with positive sensitivity to tuberculin and parotiditis pathogen.

It is important to note that the inhibiting action of aflatoxin became apparent only after 20 hrs of its incubation with the lymphocytes culture. When the incubation period was shorter, the inhibitory effect of the aflatoxin was reversible and could be interrupted by the washing off of lymphocytes with an isolation medium.

Paul R.S. et al. (1977) have also demonstrated the immunodepressive action of aflatoxin in vitro on a lymphocytes culture. Lymphocytes were isolated from cattle blood and stimulated by non-specific phytomitogens - phytohaemagglutinin, concanavalin A (Con-A), pokeweed mitogen and a specific antigen - PPD.

Aflatoxin in a dose of 10-20 mg/ml induces a 50% inhibition of labelled thymidine absorption by lymphocytes stimulated by three non-specific phytomitogens: PHA, Con-A, PWM. Peripheral bovine lymphocytes infected by cattle TB pathogens - Mycobacterium bovis - were stimulated by PPD. These lymphocytes displayed a 50% inhibition of labelled thymidine absorption under the action of aflatoxin in a concentration of 0.5 mg/ml. The viabi-

lity of lymphocytes in the culture was practically unchanged at aflatoxin B_1 concentration of 50 mg/ml.

It is to be taken into account that membrane receptors for phytohaemagglutinin, concanavalin A, and PPD are peculiar to T lymphocytes while receptors for pokeweed mitogen - to T and B cells.

Inhibition of cellular immunity reactions was observed by Richard J. et al. (1975 1978) after the introduction of stachybotritoxin to guinea pigs before BCG immunization. The animals which had received mycotoxin produced no positive skin test reactions to a later intracutaneous tuberculin injection.

It is thus evident that mycotoxins inhibit cellular and humoral immunity manifestations in man and mammals.

The effect of the immunodepressive action of mycotoxins depends on the dose, intake duration, and species of animals.

It should be noted that the peculiarities of the inhibition of immunological reactions by mycotoxins exemplified by aflatoxin B_1 are greatly determined by the biochemical mechanism of its action on cells and may be explicitly accounted for by the following characteristics:

1) The process of the damage of lysosome membranes by aflatoxin and a further release of activated hydrolases can activate the splitting of antigen and antibody macromolecules which results in inhibition of the immune response.

In this respect it should be noted that high doses of aflatoxin may cause inhibition of the RES function which provides for the preliminary treatment of the antigen (Mochael G.J. et al., 1973).

2) The interaction of aflatoxin or its metabolites with

DNA impairs DNA matrix properties and prevents DNA replication, RNA transcription, and protein synthesis.

This impairment results in the inhibition of the differentiation of T and B lymphocytes and in the suppression of the synthesis specific immunoglobulins.

3) It is important to stress that the reversibility of the aflatoxin B_1 effect on cellular and humoral immunity correlates well with the phenomenon of reversible inhibition of DNA and RNA synthesis by aflatoxin.

Such impairment of the metabolism of nucleic acids under the action of mycotoxins may serve as a basis for mutations and malignant cellular transformations.

Burnet F.M. (1971) has experimentally demonstrated that animals in an immunodeficient state are more sensitive to carcinogenic effects. In this connection the immunodepressive properties of mycotoxins may explain, however partially, an extremely high level of their carcinogeneity since the main goal of the immunological control is the destruction of genetically heterologous cells including cancer cells.

Thus, mycotoxins, and particularly aflatoxins, in fodder and food products present a threat to the immunity system of man and livestock. The immunodepressive effect of mycotoxins raises the sensitivity of the organism to viral and bacterial infections and also to malignant degeneration of cells.

Thus, the investigation of the many-sided effect of mycotoxins on the organism of animals and human health, including immunity changes, represents a significant social and medical task. The fulfilment of this task connected with the improvement of the care of public health and environment control requires continuous efforts and cooperation within the frame-

work of the scientific communities of international organizations.

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