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

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AFLATOXINS AND THEIR BIOLOGICAL ACTIVITY

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The group of aflatoxins (secondary metabolites of microscopic fungi of the Aspergillus genus)includes more than 10 compounds of similar chemical structure and biological action. It is mostly aflatoxins B_1, B_2, G_1, and G_2 that are found in natural conditions as contaminants of food products and feeds. Out of four main representatives of aflatoxins, B_1 is most toxic and, as a rule, ti is synthesized in the largest amounts.

The results of studying aflatoxins over 20 years since their discovery have shown that most mammals (including primates), birds, some species of fish, insects, and microorganisms are susceptible, in various degrees, to the toxic action of aflatoxins.

Metabolism of aflatoxins

The alimentary pathway is the main form of entry of aflatoxins in the organism, the principal and, in most of cases, the only damaged organ being the liver.

Aflatoxin B_1 is found in the liver of rats as early as 30 minutes after being administered, and its concentration in liver reaches the maximum level within two hours. After single intraperitoneal administration, approximately 20% of labelled toxin is retained in the organism of rats within 24 hours; the highest concentration of the toxin is found in the liver. Similar results were obtained in experiments on mice, hamsters, sheep, pigs, and poultry.

Experiments with labelled aflatoxin B_1 have shown that

the main pathway of withdrawal of the toxin (in an unaltered form or as a metabolite) is its excretion with bile and urine, and only a small amount is isolated with the exhaled air in the form of CO_2. Most of investigators, studying the rate of metagolization of aflatoxins in different species of animals, have found that the half-life of aflatoxin B_1 in the organism is 12-15 hours.

Experiments conducted in recent years, both in vitro, and in vivo, have shown that aflatoxins are metabolized by the same enzymatic systems as other xenobiotics. Aflatoxin B_1 may be subject to hydroxylation by mircosomal oxidases with a mixed function o give less toxic metabolites -- aflatoxins M_1, Q_1, and P_1. Aflatoxin M_1, in many species of animals, is one of the main metabolites which are found in milk and urine. Thus, it has been detected in milk of cows, sheep, and goats which had consumed feed contaminated with aflatoxin B_1.

The second possible pathway of detoxication of aflatoxin B_1 in the organism is the reduction of cyclopentenone to aflatoxicol with the participation of soluble cytosol dehydrogenases. This reaction is reversable and therefore many authors consider aflatoxicol as a "reserve" form of aflatoxin B_1 in the cell.

Finally, aflatoxin B_1, with the engagement of the same enzymatic system of the liver microsomes, can be "activated" i.e. it can be turned into compounds with a more pronounced toxicity. It is supposed that one of such active forms of aflatoxin B_1 is its hemiacetal-aflatoxin B_{2a}, the other form being its 2,3-epoxide. It is also believed that epoxidation affects

the double bond of the terminal furane ring of the molecules of the most toxic representatives of the aflatoxin family--aflatoxins B_1, G_1, and M_1, whereas aflatoxins B_2 and G_2 whose molecules do not have that double bond, possess a much lower biological activity. It should be emphasized that acute toxic effect (aflatoxin B_{2a}) and carcinogenic activity (2,3-epoxide of aflatoxin B_1) of aflatoxins are primarily associated with these "active" metabolites of aflatoxin B_1.

The 2,3-dihydrodiol of aflatoxin B_1 which is formed from the epoxide, just as other derivatives of aflatoxin B_1, may produce in liver cells conjugates with glutathione, cysteine, gluouronic and sulphuric acids and in this form be excreted from the organism with bile or urine.

Biological activity of aflatoxins

Acute aflatoxicosis

Acute alimentary toxicoses associated with the ingestion of contaminated feeds have been described in 1960 almost concurrently for turkey-poults, ducklings, pigs, and calves. The most susceptible among farm animals are 3-12 weeks old piglets, pregnant saws, and 1-6 months old calves. As for poultry, high susceptibility to aflatoxins is found in turkey-poults and ducklings; less susceptible are young pheasants while chicken are characterized by a relative resistance.

The leading clinical symptoms of acute intoxication with aflatoxins is the absence of appetite, loss of body mass, and a reduction in weight gain. It is necessary to emphasize a

rapid development of symptoms of intoxication and a high death rate among animals. Aflatoxicoses in birds are distinguished by symptoms of the damage to the nervous system, in calves by the disruption of the function of the gastro-intestinal tract, in pigs and dogs by the development of jaundice. Characteristic symptoms of acute intoxication are multiple haemorrhages and oldemas.

Aflatoxins are hepatotropic toxins, the target organ in all species of animals being the liver. Aflatoxins cause differently expressed and differently localized necroses of the liver parenchyma and also adipoge and albuminous degeneration of hepatocytes. A characteristic feature of the action of aflatoxins is rapid proliferation of the epithelium of biliary ducts.

Table 1 sums up some data on the action of aflatoxin-contaminated feed on farm animals and poultry.

Changes in the liver, similar to those observed in farm animals, are found in experimental conditions in most of test animals. The LD_{50} values for some species are shown in Table 2. Depending on the susceptibility to aflatoxin B_1, the animals may be sorted into three groups: 1) very susceptible for which $LD_{50} \leq 1$ mg/kg; 2) susceptible for which LD_{50} is 1-10 mg/kg; and 3) resistant for which $LD_{50} > 10$ mg/kg.

It should be noted that aflotoxin B_1 is most active among aflatoxins. The relation between the toxicities of individual representatives of this group may be demonstrated on an example of LD_{50} values for one-day old dicklings, which are 0.36, 1.70, 0.78, and 2.83 mg/kg, for aflatoxins B_1, B_2, G_1 and G_2, respec-

tively. LD_{50} of aflatoxin B_1 for rats of the Fischer line is 1.16 mg/kg, and that of aflatoxin G_1 is 1.5-2.0 mg/kg; aflatoxins B_2 and G_2 are weakly toxic at doses in excess of 200 mg/kg

Information on the toxic action of aflatoxins upon primates is of definite interest. It has been demonstrated in experiments on rhesus monkeys and crabeaters that aflatoxin B_1 in doses ranging from 62 μg/kg of the body mass to 5 mg/kg induces changes in the liver which are characteristic of aflatoxicosis and rapid (at high doses) death of animals. A special syndrome has been described in young Macaca fascicularis females after the internal administration of aflatoxin B_1 in a dose of 0.5 or 1.5 mg/kg. The characteristic clinical symptoms were caughing, vomiting, diarrhea, and coma. The changes in liver included centrilobular necrosis, moderate proliferation of biliary ducts, and massive adipose degeneration which was also observed in the heart and kidneys. Oedema of the brain and the degenerative changes of nerve cells were also observed. Some of these changes were similar to symptoms noted in children who suffered from Reye's syndrome which will be described later on. Thus, the sensitivity of monkeys to acute action of aflatoxins is indubitable.

As seen from Table 1 and 2, there are considerable interspecific differences in susceptibility to aflatoxins. The reason, according to many investigators, is due to the differences in the rates of aflatoxin metabolization between the species; other authors believe it to be associated with different pathways of metabolism.

It is noteworthy that there are differences in suscepti-

Table 1

Toxic action of feeds contaminated with aflatoxins on farm animals and poultry

Animal	Dose	Effect
Cattle		
calves	0.08 mg/kg/day	Drop in weight gain
	0.2 mg/kg/day	Drop in weight gain, coagulopathy
	0.5 aflatoxin B_1/kg/day	Coagulopathy, necroses of liver, death
bulls	0.7 mg/kg feed	Drop in weight gain
	1.0 mg/kg feed	Death (59 days)
adult cows	2.0 mg/kg feed	Drop in milk yield
	16.0-46.0 µg/kg	Detection of aflatoxin M_1 in milk
	0.6-0.9 mg/day/cow	Detection of aflatoxin M_1 in milk
Horses	0.075 aflatoxin B_1 kg/day	Disruption of liver function, jaundice, death on the 37th day
	0.15 mg/kg/day	Ditto, death on 26th day
	0.3 mg/kg/day	Ditto, death on 12-15th day
Pigs		
mass 6.5 kg	0.62 mg/kg	Corresponds to LD_{50} (internally)
mass 20 kg	0.26 mg/kg feed	Growth retardation
	0.065 mg/kg/day	Suppression of immunogenesis
mass 22 kg	2 - 4 mg/kg feed	Acute toxicosis, death
Poultry		
chicken	0.25 mg/kg feed	Drop in weight gain
	1.0 mg/kg feed	Necrosis of the liver, death
broilers	0.25 mg/kg feed	Suppression of immunogenesis
	0.6 mg/kg feed	Drop in resistance
	1.5 mg/kg feed	Drop in weight gain
	2.5 mg/kg feed	Coagulopathy
	5 - 10 mg/kg feed	Necrosis of the liver, death
laying hens	2 - 8 mg/kg feed	Reduction in egg laying capacity
	20 mg/kg feed	Reduction in egg-laying capacity, detection of aflatoxin in eggs

Table 2

Values of LD_{50} of aflatoxin B_1 for some species of farm and laboratory animals (single administration)

Animal	LD_{50}, mg/kg of body mass
Ducklings	0.34-0.56
Rabbits	0.3 -0.5
Rainbow trout	0.5
Cats	0.55
Mink	0.5 -0.6
Pigs	0.62
Dogs	1.0
Guinea pigs	1.4 -2.0
Sheep	2.0
Monkeys	2.2
Rats:	
newborn	0.56
weanlings	5.5
adult males	7.2
adult females	17.9
Chicken	6.5 -16.5
Mice	9.0
Hamsters	10.2
Chicken embryos	0.025 μg/egg

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bility to aflatoxins even among breeds of one and the same species. For instance, the study of 18 strains of chicken, turkey-poults and quail indicated that only one breed -- New Hampshire-- is distinguished by high susceptibility to aflatoxin B_1. A comparison of the susceptibilities of the embryos of different breeds of hen to aflatoxin B_1 revealed that most resistant to this aflatoxin are Rhode Island embryos and the least susceptible are the embryos of the White Plymouthrock breed.

In vitro studies of different systems have greatly facilitated the elucidation of the biological activity of aflatoxins. Thus, toxic properties of aflatoxins have been proved in relation to cultures of chick embryo liver, baby rat liver, lungs, and kidneys, calf and monkey kidneys, human liver, etc. In these systems, the activity of aflatoxin B_1 was demonstrated at a dose ranging from 0.01 to 10 µg/ml. Its toxicity was expressed in the changes in the morphology of cultivated cells and in the disruption of their functional activity: suppression of the synthesis of nucleic acids and protein.

The information about high susceptibility of tissue cells of man --liver, lungs, blood cells, and skin fibroblasts --to aflatoxins is of interest. Along with aflatoxin B_1, toxic action on the cells of human embryo liver is exerted by aflatoxins B_2, G_2, and G_1.

Numerous observations and experiments have indicated that aflatoxins are strong immunodepressants, which mostly affect cellular immunity. The T system of cellular immunity is distinguished by extremely hig susceptibility to aflatoxins

B_1 and M_1. Aflatoxins also influence the mechanisms of non-specific resistance of the organism -- the synthesis of some fractions of the complement, the production of interferon, etc.

Chronic aflatoxicosis

Chronic intoxication with aflatoxins entails the development of malignant tumours of the liver. At present, aflatoxins, primarily aflatoxin B_1, are classed with the strongest chemical cancirogens.

When administered internally, aflatoxins induce hepatomas in all thus far studied species of animals, specifically in rats of the Fischer, Wistar, and Porton strains, ducklings, chicken, rainbow trout, salmon, guppies, pole cats, dogs and monkeys.

A linear dependence of the frequency of hepatocellular carcinomas upon the dose of aflatoxin B_1 in the ration was observed in rats of the Fischer strain: at aflatoxin concentration of 1 μg/kg the frequency of tumours was 10%, at a concentration of 100 μg/kg it was 100% (Table 3). The frequency indices of cancer in the lifetime of rats, theoretically calculated by the results of various experimental studies, were $240/10^5$ at a concentration of aflatoxin B_1 in the ration 0.1 μg/kg and $1100/10^5$ rats at a concentration of 0.3 μg/kg of feed.

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Table 3

Dependence of carcinogenic activity of aflatoxin B_1 in male rats of the Fischer strain upon its content in the ration

Aflatoxin concentration, µg/kg	Duration of feeding, weeks	Frequency of liver carcinoma	Earliest time of the development of the tumour, weeks
0	74 - 109	0/18	-
1	78 - 105	2/22	104
5	65 - 93	1/22	93
15	69 - 96	4/21	96
50	71 - 97	20/25	82
100	54 - 88	28/28	54

Single intraperitoneal administration of aflatoxin B_1 in female rats at a dose corresponding to LD_{50} also led to the development of hepatomas in seven out of 13 rats within 60-128 weeks.

Carcinogenic action of aflatoxins may manifest itself also in the progeny: the development of cholangiocarcinomas has been observed in baby rats subjected to prenatal (intrauterine) or post-natal (through mother's milk) exposure to aflatoxin B_1.

There are reports about the development of tumours outside the liver as a result of the administration of aflatoxin B_1: carcinomas of the stomach, adenocarcinomas of the large

intestine, kidnwys and lungs, tumours of the salivary glands, tongue, and oesophagus. The cases of the development of sarcomas in the place of subcutaneous administration of aflatoxin are also described.

Carcinogenic properties of other aflatoxins --B_2, G_1, G_2, M_1 are less pronounced. When aflatoxin G_1 was administered to rats with dringking water it induced not only tumours of the liver but also tumours of the kidneys. Aflatoxin B_2 in a total dose of 150 mg per animal induced hepatocellular carcinomas in three out of nine rats within of 57-59 weeks. In the same series of experiments, aflatoxin B_1 administered in a dose of 1.3 mg per animal induced hepatomas in 9 out of 9 rats within 46 weeks. These results indicate that the effective dose of aflatoxin B_2 is 115 times higher than the dose of aflatoxin B_1 giving rise to hepatomas in rats.

Unlike rats, mice manifest well-pronounced resistance to the carcinogenic action of aflatoxins administered internally. Prolonged consumption of aflatoxin B_1 by mice at a concentration up to 1000 μg/kg of the feed failed to induce any tumours. At the same time when aflatoxin B_1 was intraperitoneally administered to baby mice in a dose of 1,25 μg/kg during the first 7 days of life or in a dose of 6 μg/kg during three days, hepatomas were found within 80 weeks (Table 4).

Rainbow trout is known for high susceptibility to aflatoxins. The inclusion of aflatoxin B_1 into its feed at a rate of 0.1 μg/kg induces the development of hepatomas within 20 months. Aflatoxin M_1 manifests itself as a weaker hepatocarcinogen in experiments with trout (Table 5).

Table 4

Carcinogenic activity of aflatoxin B_1 in different species

Species	Dose	Duration of observation	Tumor formation frequency (%%)	
Ducklings	30 µg/kg of the ration	14 months	72	(8 of 11)
Trout	8 µg/kg of the ration	1 year	40	(27 of 65)
	4 µg/kg of the ration	1 year	15	
Rhesus monkeys	100 - 800 mg (total dose)	over 2 years	7	(3 of 42)
marmosets	5.0 mg (total dose)	2 years	66	(2 of 3)
tupaias	24 - 66 mg (total dose)	3 years	75	(9 of 12)
Rats	100 µg/kg of the ration	54 - 88 weeks	100	(28 of 28)
Mice	150 µg/kg of the ration	80 weeks	0	(0 of 60)
	1000 µg/kg of the ration	80 weeks	0	(0 of 30)
newborn	6.0 µg/g of body weight (3 doses intraperitoneally)	80 weeks	100	(16 of 16)
Pole cats	0.3 - 2.0 µg/kg	28 - 37 months	78	(7 of 9)

Table 5

Hepatocarcinogenecity of aflatoxin M_1 in rainbow trout as compared to aflatoxin B_1

Aflatoxin level in the ration, µg/kg	Liver tumor frequence	
	males	females
M_1 4.0	4/28 (14%)	13/27 (48%)
M_1 16.0	22/27 (81%)	11/14 (79%)
M_1 32.0	24/25 (96%)	13/14 (93%)
M_1 64.0	21/24 (88%)	9/10 (90%)
B_1 4.0	15/22 (68%)	18/23 (78%)

For a long time (up to 1971) primates were believed to be resistant to carcinogenic action of aflatoxins. It was only in 1972 that Gopalan and collaborators published a report about the development of hepatocellular carcinoma in a male rhesus monkey which had been fed with partially purified aflatoxins B_1 and G_1 for 5.5 years; the carcinoma was detected 8 years after the commencement of the experiment. Furtheron, these results were repeatedly confirmed by other authors. The hepathocarcinogenic effect of aflatoxins B_1 and of a mixture of aflatoxins $B_1 + B_2 + G_1 + G_2$ has been demonstrated for marmosets and tupaias of both sexes. It should be noted that attempts at determining the dose dependence of the effect have not been undertaken for primates.

Teratogenic and mutagenic action of aflatoxins

Teratogenic properties of aflatoxin B_1 have been determined in experimental conditions for hamsters, rats, mice, chicken, and Japanese smooth snake (Oryzius latipes).

Aflatoxin administered to hamsters on the 8th day of pregnancy induced malformations in 29.4% of foetuses. The administration of aflatoxin B_1 at a concentration of only 1.0 μg/kg to female wistar rats every other day for the first 14 days of pregnancy entailed the death of 1% of embryos, resorption of 6.8% of embryos, while 3.5% of fetuses had various malformations of development: microcephalia, hernias, bradidactylias.

In 11.5% of mice embryos exposed to aflatoxin B_1, at the 8th day of intrauterine development various malformations were observed: brain hernias, anomalies of the gastrointestinal tract.

The administration of aflatoxin B_1 to the yolk sack of hen eggs at the 6th day of incubation entailed the development of malformations in 65-90% (depending on the dose) of embryos.

Aflatoxin B_1 induces chromosomal aberrations and breakups of the DNA in plant and animal cells. It has been likewise demonstrated that it produces mutations of genes in bacterial test-systems (the Ames test) after metabolization ("activation") by microsomal preparations from the rat or human liver.

Studies of recent years have demonstrated that mutagenic and toxigenic properties of the precursors of aflatoxin B_1 (during its biosynthesis) increase as their structure becomes more complex and reach their peak in aflatoxin B_1. The depend-

ence of mutagenic properties of aflatoxin B_1 upon the presence of the lactone ring in its structure has been demonstrated.

Factors influencing the biological activity of aflatoxins

A large amount of data accumulated till now demonstrates the possibility of modification of toxic, carcinogenic, and other manifestations of the biological activity of aflatoxins within broad limits by using different factors.

The toxic action of aflatoxins depends considerably upon the age and sex of animals. The common feature for all species is a decrease in their susceptibility to aflatoxins with age. As was seen in Table 2, LD_{50} for newborn rats is almost 1/10 of that for weaned rats and is 1/13 of that for the adult male rats.

Numerous studies have demonstrated that females are more resistant both to the acute toxic and to the carcinogenic action of aflatoxins compared to males. Table 2 also shows that adult male rats are approximately 2.5 times more susceptible to aflatoxin B_1 than females (LD_{50} 7.2 and 17.9 mg/kg of body mass, respectively). It is not without interest that when aflatoxin B_1 is included in the rations of rats of both sexes, the frequency of pre-cancer changes in the liver is practically the same for males and females, but the period between the appearance of these changes and the development of hepatic carcinoma in females is much longer than in males. Table 6 gives the estimated figures of the total amounts of aflatoxin B_1 enter i the organism of animals (male and female rats) in the period preceding the appearance of the liver tumours.

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Table 6

The dependence of carcinogenic activity of aflatoxin B_1 upon sex of rats

Concentration of aflatoxin B_1 mg/kg of the ration	Total dose of aflatoxin B_1, mg/kg animal		Time of detection of the liver tumours, days	
	males	females	males	females
1.0	2.9	5.9	245	448
0.015	0.095	0.115	476	560

There are all grounds to believe that the revealed sex distinctions in the susceptibility of animals to aflatoxins are determined by differences in the hormonal background. The administration of diethylstilbestrol concurrently with aflatoxin B_1 to male rats entails considerable lowering in the frequency of liver tumours (8 out of 40 as against 25 out of 35 in the control). The development of tumours in the liver in male rats under the influence of aflatoxin B_1 was prevented by a preliminary hypophysectomy and also by castration of the animals. The administration of testosteron along with aflatoxin B_1 to castrated rats entailed death of all experimental animals.

There is special interest in the information about the influence of the factors of nutrition upon the biological activity of aflatoxins. It has been demonstrated that under the conditions of protein insufficiency the frequency of tumours of the liver under the action of aflatoxin B_1 is much higher than against the background of full-value nutrition and the time of the development of tumours is considerably less.

Thue, in rats which were fed on rations containing 9% of protein, tumours of the liver developed in 11 out of 15 animals after the passage of 8 months whereas in rats which consumed rations with 22% of protein tumours developed in 7 out of 14 animals within 10 months . In the case of protein insufficiency, the acute toxic effect of aflatoxins is also more pronounced. At the same time there is a different information indicating a decrease in the carcinogenic effect of aflatoxins under the conditions of protein deficiency in the rations. It was shown that not only protein insufficiency but also the deficiency of certain amino acids (tryptophan, specifically) may aggravate aflatoxicosis.

Speaking of other alimentary factors capable of modifying the biological activity of aflatoxins, we should single out lipotropic agents, some vitamins, and microelements. The insufficiency of methionine and choline in the rations has a protective action in relation to the acute toxic effect of aflatoxin B_1, but reliably intensifies its carcinogenic activity in experiments on rats. At the same time, a more pronounced deficiency of these lipotropic substances in the rations rather decreases, than increases, the frequency of hepatocarcinomas in rats which were given aflatoxin B_1. It should be likewise stressed that the effect of lipotropic substances which modify carcinogenic activity depends largely upon the amount and quality of fats in the ration of experimental animals.

The final biological effect of aflatoxins is strongly influenced by the availability of vitamins. For instance, the deficency of vitamins A and C produces inhibition of the meta-

bolism of aflatoxin B_1. Some authors stress that whenever there is a deficiency of vitamin A, there is a greater frequency of carcinomas of the large intestine in rats.

Pronounced protective action in relation to aflatoxins has been demonstrated by selenium and copper added at definite concentrations to the rations of rats.

Finally, attention should be drawn to the activation of microsomal oxidases with a mixed function under the influence of ethanol which may intensify the formation of the "active" form of aflatoxin B_1 its 2,3-epoxide-- and increase thereby the hepatocarcinogenic activity of aflatoxin B_1.

It seems that most of factors modifying the aflatoxin toxicity act by changing the activity of enzymatic systems which metabolize aflatoxins. This supposition is confirmed by the results of study of the influence of inductors and inhibitors of microsomal oxidases with a mixed function upon the biological activity of aflatoxins. Thus, when test animals are given phenobarbital which induces the activity of oxidases, the toxic effect of aflatoxin B_1 decreases which manifests itself in the reduction of the inhibiting action of the toxin on the synthesis of the protein, the prevention of the liver necroses, the weakening of aflatoxin's carcinogenic properties. At the same time, the administration of an inhibitor of microsomal oxidases (SKP525A) was accompanied by an intensification of the damaging action of aflatoxin B_1 upon the liver.

Thus, the results of sudes of aflatoxicoses in farm animals and the experimental findings enable us to class aflatoxins with most potent hepatotoxic and hepatocarcinogenic to-

xins. It should be stressed that the extent of biological activity of these microtoxins depends greatly upon the species of animals, their age, sex, and the nature of nutrition.

Action of aflatoxins on man

Clinical observations

Since aflatoxins were found to possess strong hepatotoxic and hepatocarcinogenic properties, the relatively high frequency and level of the contamination of food products with aflatoxins and the abundance of aflatoxin producer in natural conditions make us to class these microtoxins with biological pollutants of the environment which are potentially dangerous for man's health.

Acute aflatoxicoses in humans are rare and are associated with high concentrations of aflatoxins in food (ranging from 0.2 to several mg/kg). All cases of poisonings occurred in countries distinguished by a high level of contamination of food products with aflatoxins (Table 7). The sickness of children in Senegal was caused by peanut flour containing aflatoxin at a concentration of up to 1.0 mg/kg. In India, children in the age group from 1.5 to 5.0 years when treated for the syndrome of protein insufficiency -- kwashiorkor--were given peanut flour which contained aflatoxin B_1 at a concentration of 0.3 mg/kg. The average daily dose of the toxin in this case was 1.1 μg/kg of body mass. In one of described cases, the liver of a 15 year-old boy which died of acute hepatitis demonstrated changes which are characteristic of aflatoxicosis.

Table 7

Cases of liver diseases in humans associated with the ingestion of food contaminated with aflatoxins

Country	Number of liver disease cases	Age group	Contaminated food product	Concentration of aflatoxins, mg/kg	Clinical symptoms of aflatoxicosis
Senegal	2	4-6 years	peanut flour	0.5-1.0	Hepatitis; in one case fibrosis of the liver
China	26	all ages	rice	0.2	Acute hepatitis; in three cases (children) lethal outcome
Uganda	1	15 years	manioc	1.7	Acute hepatitis with lethal outcome
India	20	1.5 years-5 years	peanut flour	0.3	Hepatomegalia, insufficiency of the liver, in three cases lethal outcome; development of cirrhosis of the liver
	400	adults	maize	0.25-15.0	Acute hepatitis. Lethal outcome, more than in 25% of all cases

The disease was caused by manioc containing aflatoxin B_1 at a concentration of 1.7 mg/kg.

A convincing instance of the association of aflatoxins with acute hepatitis in people was the outbreak of toxic hepatitis in the North-West districts of India in 1974. The disease was characterized by a subacute commencement with a fever an a subsequent rapid development of jaundice (98% of cases) and ascitis. The patients demonstrated hepatosplenomengalia. Liver section obtained by biopsy and autopsy demonstrated a characteristic proliferation of biliary ducts. The death rate was very high. The analysis of food products indicated that the cause of the disease was maize which contained aflatoxin B_1 at a concentration of up to 15.6 µg/kg. With this level of contamination, the daily ingestion of aflatoxin ranged from 2 to 6 mg/per person which corresponds to daily doses of up to 120 µg/kg of body mass.

Special mention should be made of information about the influence of aflatoxin upon man in industry. In one of the instance, 7 people out of 55 who were engaged in the processing of peanuts and other oil-bearing crops, demonstrated the development of cancer of varying localization (the period of observation was 11 years, the time of exposure was 2-3 years). The concentration of aflatoxins in air in this case could have been in a range of 0.87 to 72 mg/m^3. Two cases of pulmonary adenomatosis with a lethal outcome have been described in people handling Brazilian peanuts. Changes characteristic of the action of aflatoxin B_1 were found in their lungs. Finally, a report is known about finding carcinoma of the large intestine

in two research workers who for a number of years were engaged in the isolation and purification of aflatoxins.

The study of patients suffering of cirrhosis of the liver, residing in the regions of Iran where this disease is believed to be frequent, revealed the presence of aflatoxin M_1 in the urine of six out of 26 patients. Aflatoxin was never found in the urine of patients with other diagnoses. The patients came to the clinic from villages which were noted for a high concentration of aflatoxin M_1 in milk.

The possible relation of Reye's syndrome with the contamination of food by aflatoxins is still debatable. Out of four countries where this relationship was studied (Thailand, New Zealand, USA, and Czechoslovakia) and where aflatoxin B_1 had been found in the liver of patients, only Thailand is situated in an area with a high frequency of contamination of food with aflatoxins. The three following reports are noteworthy.

In Thailand, the autopsy of 23 children deceased as a result of Reye's syndrome revealed considerable amounts of aflatoxin B_1 (up to 93 μg/kg) in the liver, in the content of the stomach and the intestines (up to 127 μg/kg), and in bile. Traces of aflatoxin B_1 have been also detected in other tissues-brain, kidneys- and in the urine.

Reports from Czechoslovakia present results of clinical observation of 27 children in the age group from 3 days to 8 years who also died of Reye's syndrome. The disease was characterized by an acute onset. In some cases, brain symptoms prevailed and the disease lasted from 2 to 3 months. In subacute cases, periportal fibrosis and the proliferation of the bile ducts were observed in the liver: when the disease con-

tinued for 4 months, there were symptoms of cirrhosis. In all cases, aflatoxin B_1 was found in liver tissues at a concentration of from 20 to 2760 μg/kg; in three cases aflatoxin M_1 was also found.

In 1979 Rayan and collaborators published results of an analysis of aflatoxins in the liver of 8 children with Raye's syndrome. In six cases, the concentration of aflatoxin B_1 ranged from 2.23 to 17.33 μg/kg of the tissue. In two children, during the acute stage of the disease, the aflatoxin was found also in blood at a concentration of 11.93 and 31.3 mg/ml. There are reports of other investigators about cases when aflatoxin B_1 was found in blood serum of patients suffering from Reye's syndrome.

Thus, available information makes it possible to conclude that aflatoxins may play a definite part in the development of Reye's syndrome in humans in some areas. At the same time we cannot exclude the possibility that the pathological changes peculiar to Reye's syndrome, may lead to the disruption of metabolism and elimination of aflatoxins, thereby retaining them in the organism.

Epidemiological studies

The results of studying a possible correlation between the level of contamination of food products with aflatoxins and the frequency of primary cancer of the liver in humans are of considerable interest.

Primary cancer of the liver in European countries comprises 1.2 per cent of all cancer cases, in USA 2.5-2.8 per cent, in African countries 14%. The highest frequency of this disease

is observed in Bantu males in South Africa (Mozambique): up to 68-77% of all cancer cases.

Epidemiological studies indicate a correlation between the level of daily ingestion of aflatoxins and the frequency of primary cancer of the liver in some parts of Kenya, Mozambique, Swaziland, and Thailand (Table 8).

Most interesting are the data about the age distribution of the frequency of primary cancer of the liver among the population of Mozambique. It is noteworthy that in areas with a high frequency of this disease the peak is found in the early age period -- 20-29 years, whereas in areas with a low frequency of primary cancer of the liver, the morbidity increased with age gradually. This pronounced "juvenation" of primary cancer of the liver may be explained by a greater susceptibility of a growing organism to the carcinogenic action of aflatoxins.

Some authors believe that the virus of hepatitis B which is widely spread in countries with a high incidence of primary cancer of the liver may also act as a cofactor in the etiology of this disease.

In studies undertaken in Indonesia on 71 patients suffering of primary cancer of the liver, aflatoxins were found in biopay samples of the liver in 57.7 cases. Anamnestic data indicated the consumption of contaminated food products, including a prolonged daily consumption of peanuts. Aflatoxin B_1 was found in samples of food products at concentrations ranging from 17 to 1190 µg/kg while aflatoxin G_1 was found at concentrations ranging from 5 to 630 µg/kg.

In the United States, we know of a case when aflatoxin B_1

Table 8

Correlation between the level of ingestion of aflatoxins with food and the frequency of primary cancer of the liver in some countries of Asia and Africa

Country	Area	Estimated consumption of aflatoxins by adults, ng/kg of body mass/day	Frequency of cancer of the liver	
			Number of registered cases	Incidence per 100,000 of the population per annum
Kenya	Montain area	3.5	4	1.2
Thailand	Sonkla	5.0	2	2.0
Swaziland	High veld	5.1	11	2.2
Kenya	Elevation	5.9	33	2.5
Swaziland	Medium veld	8.9	29	3.8
Kenya	Lowland area	10.0	49	4.0
Swaziland	Lebombo	15.4	4	4.3
Thailand	Ratburi	45.0	6	6.0
Swaziland	Low veld	43.1	42	9.2
Mozambique	Inyambane	222.1	more than 100?	13.0

at a concentration of 520 μg/kg of wet weight was found in the liver tissue of a patient suffering of carcinoma of the rectum and liver.

Published epidemiological studies are limited in volume and, besides, they evaluate but one of the possible etiological factors: the influence of aflatoxins. There are no doubts that other factors , such as malnutrition, viruses, other mycotoxins, plant alkaloids, helminthiases, may also play an etiological or a modifying part in the development of cancer of the liver.

Conclusion

Thus, the observations of alimentary aflatoxicoses among farm animals in many countries and the results of numerous experimental studies demonstrated that alfatoxins are highly toxic compounds which affect mainly the liver. Acute aflatoxicosis is characterized by the development of necroses of hepatocytes and the proliferation of the biliary ducts; chronic intoxication may entail cirrhosis of the liver and the development of hepatomas.

Aflatoxin B_1 induces chromosomal aberrations and ruptures of the DNA in plant and animal cells, and in some bacterial test systems -- gene mutations after activation with microsomal enzymatic systems. High concentrations of aflatoxins have a teratogenic action upon some species.

The biological activity of aflatoxins is dependent on the age and sex of animals. The nature of nutrition, and primarily the amounts of protein, lipotropic substances, and some vita-

mins, may materially modify the course of aflatoxicoses.

A correlation has been found between the level of aflatoxin B_1 arriving with food and the incidence of cancer of the liver in man, in areas with high frequency and high level of contamination of food products with aflatoxins and high frequency of the primary cancer of the liver.

At the same time, the number of trends in the study of biological activity of aflatoxins call for further development. It is necessary to have a more detailed information about the absorption of aflatoxins in the gastrointestinal tract and about the rate of their withdrawal from tissues. We find it important to study the modifying role of alimentary factors and of other biologically active substances in the manifestation of the toxicity of aflatoxins. This aspect should be taken into consideration also in epidemiological studies related to the connection between primary cancer of the liver in man and intoxication with low concentrations of aflatoxins.

The supposition about the casual relation between the consumption of aflatoxins and primary cancer of the liver in man and also about the role of aflatoxins in the development of Reye's sundrome requires a further substantiation.

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