AFLATOXIN RESIDUES IN EDIBLE TISSUE OF POULTRY IN ZAMBIA

by

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A dissertation submitted to the University of Zambia in partial fulfilment of the requirements of the degree of Master of Science in Biochemistry.

The University of Zambia
Lusaka, Zambia
1997

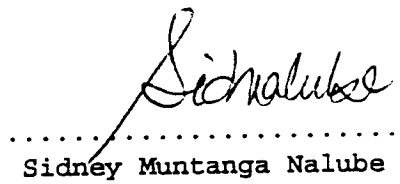
DEDICATION

Dedicated to the late John Mulife and Jones Muntanga Nalube.

Sidney.

DECLARATION.

I hereby declare that this dissertation is my own work and that it has not been previously submitted for degree purposes here or at any other University.



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APPROVAL

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ABSTRACT.

The aflatoxins, toxins produced by the fungus Aspergillus flavus, have been detected in both imported as well as locally grown maize in Zambia.

The problem of toxicity caused by the ingestion of aflatoxin-contaminated maize was approached by determining the levels of residues in edible tissue of poultry from known dietary levels of aflatoxin in maize. The livers and spleens, gizzards and hearts, and thighs were chosen for aflatoxin residue analysis.

This study was conducted to determine the aflatoxin residue levels in tissues of village chickens that had been fed diets containing total aflatoxin concentration of 4750mg/kg of feed (equivalent to aflatoxin B1 concentration of 1650 mg/kg) at 0% (for controls) 25% (for low AF diet) and 75% (for high AF diets) inclusion levels for 21 days. Residues of aflatoxin were determined by thin layer chromatography. Results show that aflatoxins B1, B2, G1, G2 and M1 were deposited in all tissues analysed. The highest levels were present in the livers and spleens (8.0 mg/kg, low AF diet; 15.4 mg/kg, high AF diet) and the lowest were in the thighs (1.6 mg/kg, low AF diet; 9.4 mg/kg, high AF diet). At the end of the experiment, the livers were observed to be pale, enlarged and friable.

Since only a small fraction (less than 1%) of the highest level of aflatoxin tested and ingested was deposited in the tissues, either as the original compound or as its metabolite, there appears to be little danger of carry-over of dietary aflatoxin to poultry tissue. The toxic levels (i.e. levels that can cause death) to humans is considered to be approximately 1.7 mg/kg daily (Campbell and Stoloff, 1974), which means that the amount the poultry would need to consume to reach this tissue level would cause pathological symptoms or even death. Therefore the chance of these contaminated birds entering the food chain can be considered to be minimal since people traditionally do not eat poultry dying of unknown cause.

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It is my pleasure to acknowledge the assistance received in the course of the preparation of this work.

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Finally, I must record my thanks to Ms Patricia Phiri, and Mainga who for three years, coped with maps, papers and samples all over the study room in a way that a few people would have done.

This dissertation was typed by Mr Alex Siyamba, a friend at Central Statistical Office, Data Processing unit.

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CHAPTER 1

1.0 INTRODUCTION.

1.1. ZAMBIA

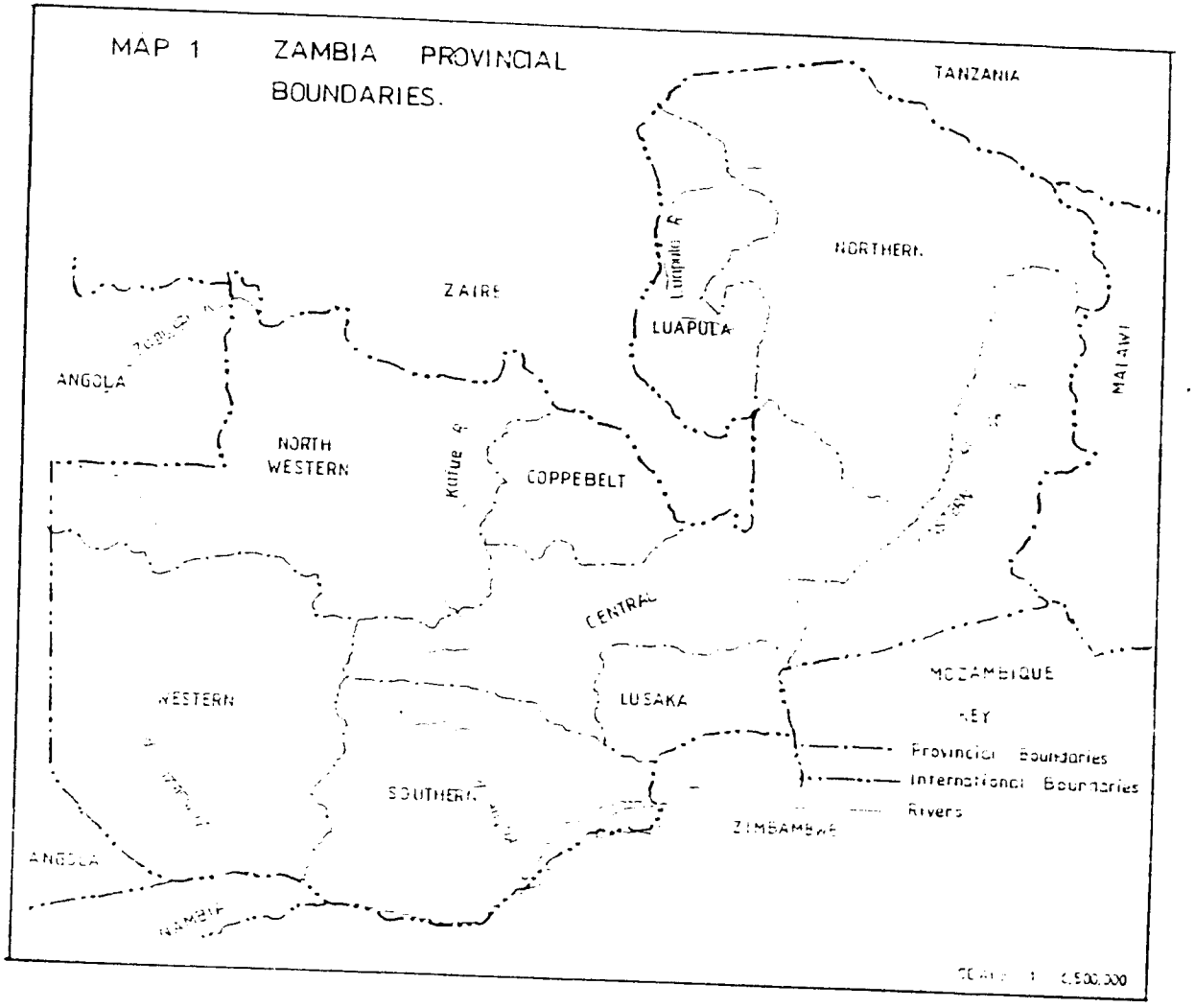
1.1.1. ZAMBIA : GENERAL.

Zambia is a large landlocked country with an area of 751,929 square kilometres and a human population of 8,500,000 (Central Statistics Office, 1992). It has borders with Angola, Namibia, Botswana, Zimbabwe, Mozambique, Malawi, Tanzania and Zaire (Map 1;Fig 1). Nearly 60 percent of the population live in rural areas giving a low population density in many parts of the country, especially in Eastern, Northern, North-Western and Western provinces. On average, Zambia has a population density of nearly 10.4 persons per square kilometre.

1.1.1.1. CLIMATE.

Zambia has a tropical type of climate because it lies between the Equator (latitude 0 degrees) and the Tropic of Capricorn (latitude 23.5 degrees South), specifically, between 9 and 18 degrees south. Being situated in the middle of the Southern African land mass, many kilometres from the ocean, the climate is continental and semi-arid. The tropical climate is tempered by an altitude of 1,000 to 1,500 meters and the general topography is in the form of a series of plateaux (Adams and Harman, 1977). Landlocked Zambia has access to the ocean via Dar-es-Salaam in Tanzania, Beira and Nacala in Mozambique, Lobito in Angola and Durban, Port Elizabeth, East London in South Africa (Map 2; Fig 2).

The tropical climate of Zambia has had a crucial influence on the storage of food. There are three seasonal divisions in Zambia. A single hot and rainy season (traditionally the planting/growing season) normally begins in October and extends, with variations in rainfall, to late March or early April. The heaviest rainfall is from December to February. The temperatures during the rainy season are normally 20-22 ^{o}C, with relative humidities around 80 percent. After the rains, the temperature drops as the cool and dry season (traditionally the harvest period) advances until June/July, when there may be frost at night in the lower lying southern parts of the country. The day temperature is around 15-17 ^{o}C. August becomes warmer and during September and October, there is a hot and dry season with temperatures of 25 to 33 ^{o}C, and relative humidities of around 40 percent, (Njapau et. al, 1993).


MAP 1 ZAMBIA PROVINCIAL BOUNDARIES.
TANZANIA
NORTHERN
LUAPULA
ZAIRE
ANGOLA
MALAWI
NORTH WESTERN
COPPEBELT
CENTRAL
LUSAKA
WESTERN
SOUTHERN
MOZAMBIQUE
ZIMBAMBWE
ANGOLA
NAMBIA
KEY
Provincial Boundaries
International Boundaries
Rivers
1 : 1,500,000

1.1.1.2. GEOGRAPHICAL.

Zambia has nine geographical provinces: Central, Copperbelt, Northern, Eastern, Luapula, Lusaka, North-western, Southern and Western. These are further divided into 64 districts for easy political administration. The Eastern, Northern and Southern provinces are the termini for long-distance trade routes within Central and Southern Africa. Lusaka and Copperbelt Provinces have become the most developed industrial regions with a high density of population while Central, Eastern and Southern have become the most developed farming regions. The other provinces - Luapula, Northern, Western and North-Western - have become less marginal, in trade, but they continue to have little economic activity other than subsistence farming.

1.1.1.3. SOUTHERN AFRICA.

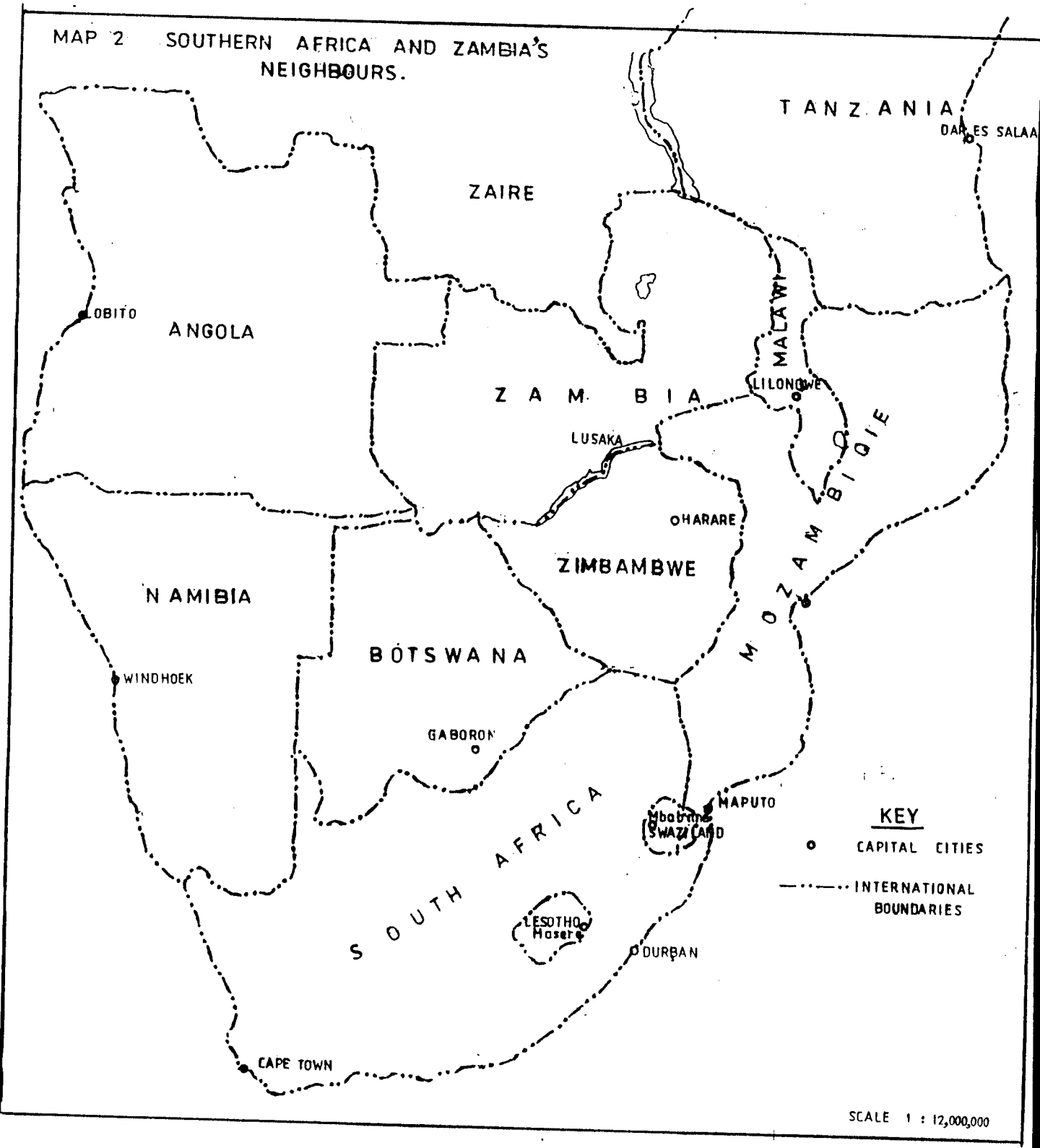
Southern Africa consists of 10 countries that is Zambia, Angola, Botswana, Lesotho, Malawi, Mozambique, Namibia, South Africa, Swaziland and Zimbabwe, (Map 2; Fig.2.). Tanzania is included because it is involved in the transportation of the relief maize to Zambia. Tanzania is normally considered part of East Africa together with Uganda and Kenya.

According to the United Nations, all Southern African countries, except South Africa, are in the group of the least developed countries with six (Zambia, Botswana, Zimbabwe, Malawi, Lesotho and Swaziland) being completely landlocked. About 60 - 70 percent of the population live in rural areas where they are engaged in subsistence production (SAPEM Magazine, August 1991). However, some countries ie. Angola, Botswana, Namibia and South Africa have performed well economically.

Southern Africa has long been a livestock rearing and crop growing region with the biggest number of animals and poultry found in rural areas. In the animal category emphasis has been placed on the cattle industry leading to the marginalisation of goats, pigs and sheep. In the poultry category, the chicken and Guinea fowl have dominated the industry. This could be due to their ease of adaptability in the rural areas. In Zambia, about 60 -70 percent of the total livestock and poultry are kept in the rural areas. The small scale farmers constitute 75 percent of the farming community and produce much of the nation's food. Commercial animal and crop production have also existed in Zambia since the settlement of Europeans.

1.1.2. SOILS AND CROPS IN ZAMBIA.

Arable soils are widespread, varying from clay to sandy soils with fertility levels being lower in high rainfall areas whose soils are heavily leached. These different types of soils have a definite effect upon the pattern of farming. Maize and cotton, for example, prefer clays since these have a high inherent fertility and can hold moisture for longer periods. Sandy soils which are well drained and aerated, on the other hand, are well suited for tobacco.


MAP 2 SOUTHERN AFRICA AND ZAMBIA'S NEIGHBOURS.
TANZANIA
DAR ES SALAA
ZAIRE
LOBITO
ANGOLA
MALAWI
LILONGWE
ZAMBIA
LUSAKA
MOZAMBIQIE
HARARE
ZIMBAMBWE
NAMIBIA
BOTSWANA
WINDHOEK
GABORON
MAPUTO
Mbabane
SWAZILAND
SOUTH AFRICA
LESOTHO
Maseru
DURBAN
CAPE TOWN
KEY
CAPITAL CITIES
INTERNATIONAL BOUNDARIES
SCALE 1 : 12,000,000

1.1.3. WATER RESOURCES IN ZAMBIA.

Zambia has large supplies of water mainly received from rains. It has several lakes, rivers and streams. Among the large rivers, are the Zambezi, Kafue, Luangwa and Chambeshi while the country has four natural lakes, namely Lakes Tanganyika, Mweru-Wantipa, Mweru and Bangweulu. These lakes are the major fishing grounds. Other fishing grounds include the Lukanga swamps, the four major rivers and the two man-made lakes (Kariba and Itezhi-tezhi). The water resources on the major rivers are used mainly for home use, transport, hydroelectricity, fishing and to a lesser extent, agriculture (irrigation) and recreation.

1.2. FARMING IN ZAMBIA.

In Zambia, Farming is usually done under the management of one person who hires labourers to help him or under the control of a family. Family members, however, do the farming themselves in the rural parts of Zambia on the small subsistence farms. Among the animals kept are cattle, pigs, sheep and goats. The different poultry include chickens, ducks and the domesticated guinea fowl. The major crops grown in Zambia are maize, groundnuts, rice, cassava, soyabeans, beans, potatoes, millet, sunflower and cotton. Maize and groundnuts are grown on both a subsistence and commercial scale in Central, Eastern, Lusaka and Southern Provinces. These provinces have a large concentration of both commercial and peasant (small-scale) farmers because of the good arable land.

Cattle farming is also very important in Zambia and provides milk, hides, manure and beef. Poultry is kept widely, on commercial farms and in villages for meat and eggs, while fish farming is practised in a few areas. In most areas, a farmer grows both commercial and food crops while raising animals and poultry.

1.2.1. FACTORS WHICH INFLUENCE FARMING IN ZAMBIA

Farming in Zambia is influenced by a number of factors, particularly the amount of rainfall received in the area and the soil fertility. These factors usually determine what type and pattern of farming is to be carried out. Rainfall is a crucial factor in determining crop yields in any season. While droughts cause failure, too much rain can also cause problems (Kadzombe et al, 1983). For example, rain at harvest time can damage crops, particularly cotton, maize and groundnuts. Easy road communication encourages farming as the produce is easily transported to the market. In Zambia, easy road communication is not available in most rural areas, which has discouraged rural farmers to produce more. Virtually all the rural population is engaged in some farming. It is estimated that about 80 - 87 percent of the total production in the rural areas is for home consumption.

1.2.1.1. ECONOMIC GROUPS OF FARMERS IN ZAMBIA.

Farmers have been put in three economic groups. There are small-scale farmers who grow only enough for their home consumption and who are found in rural areas; improved or emergent farmers who grow crops for both

consumption in the home and for sale; and the commercial farmers who use advanced farming implements and grow crops and raise animals mainly for sale. The commercial farmers are mainly found along the line of rail i.e. from Livingstone to Ndola and in areas where there are good roads and markets.

The railway line starts from Livingstone, through Lusaka and Central Provinces, to the Copperbelt Province and has since been extended with the introduction of the Tanzania Zambia Railway (TAZARA), linking the Northern Province. Other provinces i.e. Eastern, Luapula, Western and North-Western, are not linked with the railway system.

Although maize and other agricultural crops are mainly grown in four provinces, it is true to say that they are grown throughout the country in varying amounts. There are five major cereals grown in Zambia. Of these maize is consumed a great deal by over 80 percent of the population in the country. Maize consumption can be divided into direct human consumption and commercial use. It is estimated that of the maize entering official marketing channels, 83 percent is for food, 10 percent for beer, 7 percent for stockfeed and a negligible amount for food processed cereal foods. Of the amount retained on the farm by farmers, 10 percent is for animal and chicken feed (Siame and Lovelace,1989).

In 1992, Zambia had a large livestock population of over 18 million. There was a large cattle population of approximately 3 million, 257,000 of which were commercial dairy cows. Zambia had 80,000 sheep, 420,000 goats and 221,000 pigs. There were, in addition 14 million poultry birds (Nambota et.al.1992; Ministry of Agriculture, Department of Livestock Services). Of these poultry birds, the chicken was the most important. The chicken was chosen as the experimental bird for this study because of its importance in Zambian rural life.

1.2.2. LIVESTOCK FARMING

Poultry farming plays an important role in the rural economy of Zambia (Ministry of Agriculture and Water Development, Annual Report, 1987). Livestock and poultry offer rural farmers a ready source of cash for such things as food, clothing, school fees and marriage expenses. Many farmers rely on their livestock during the dry season when they have no money from crops. It is also considered important to have animals for special occasions. On the small farmer's land, animals such as oxen are used for ploughing, cultivation of crops and pulling of carts for transport of produce and inputs.

Perhaps most importantly, animals and poultry provide the family in the village with nutritious food in the form of meat, milk and eggs. Eggs are usually given to the younger ones as a source of the much needed protein and because they are easy to eat (however, in some African communities, children are forbidden from eating eggs). Meat, besides providing food, can also be a source of revenue. According to a 1988 report by the International Livestock Centre for Africa, meat from sheep and goats represented 30 percent of the meat consumed in Africa and constituted an important market for the poor farmers.

1.2.2.1. MARKETING OF LIVESTOCK IN ZAMBIA.

In Zambia, cattle and goats from rural areas travel long distances by road or rail to be sold for meat to butchers in town especially in Lusaka and the Copperbelt. Because of the inefficient transport systems and the long distances covered, the animals and sometimes poultry are likely to lose mass which means less money for the farmers as they are sold per weight. Much of the meat is marketed in urban areas where it fetches more money. Chickens and goats are the main source of meat in the village. Since the animals and poultry (in rural areas) are generally not properly looked after (poor grazing and nutrition, poor health care), their meat is tough and poor and milk production is low (Kadzombe et.al.,1983).

In 1968, livestock slaughter, dairy, poultry and other products made up 29.3 percent of the gross value of marketed products in Zambia (Kadzombe et.al.,1983). Cattle are not often used as meat source in the village as they play a very important social role. They mean wealth among the Tonga and Ila communities of the Southern province. The larger the herd owned by an individual or family, the higher the prestige he/they command in the community. The animals provide the manure used to raise the level of fertility of the soils in the fields, thereby increasing the output of crops especially in the face of escalating fertilizer costs that the rural farmer cannot afford.

1.2.2.2. POULTRY MANAGEMENT.

Poultry farming was predominantly a rural enterprise in Zambia but has become commercialised on big farms. Traditionally, it is believed that poultry can find their own food requirements and this has meant that they receive scant care and attention in the villages. In spite of this, they are still considered a valuable member of the rural farmer's household. Yet, seasonal insufficiencies of food can have adverse effects on poultry. At such times, farmers may assist by feeding the chickens damaged and rotten maize that is considered unfit for human consumption.

Some of the advantages of the common village chickens are their survival ability, their economic productive capabilities in often harsh environments (during drought and food shortage) and their power to transmit these characteristics to their offspring. It should be possible to intensify village chicken production in the remote rural areas of Zambia to help raise the quality of food and ultimately life. The majority of poultry in Zambia are kept by rural farmers under simple management procedures. There are three possible ways of management under village conditions (Barrass, 1974):

1.2.2.2.a. **EXTENSIVE MANAGEMENT.**

In many rural areas where village chickens are kept in Zambia, they are raised as scavengers and roam freely both day and night with no shelter provided. Sometimes, they shelter under a roof or verandah or under the maize storage bins at night or during the hot weather. Poultry in the village is generally left to scratch its living to supply all the nutrients. Water may be supplied to them in curved wood or broken cooking pots or dishes.

1.2.2.2.b. **SEMI-INTENSIVE MANAGEMENT.**

In this system of management, poultry are left loose in the open yard during the day and are shut in a house or shelter at night. This system, and the extensive method, are considered the most convenient as in the open, the birds have sufficient free exercise. They can also have easy access to grasses, insects, worms etc to get a balanced diet.

1.2.2.2.c. **INTENSIVE MANAGEMENT.**

Under this system of management, poultry are kept confined in a shelter at all times. The intensive system of management is convenient for keeping flocks of poultry on a big commercial basis. By adopting this system, loss of birds due to predators is avoided. However, diseases can spread quickly in crowded conditions.

1.2.2.3.d. **DISEASES OF POULTRY.**

Maintaining the health of poultry populations is important. It helps to ensure good quality products for the consumer and reduces the possible transmission of diseases between the birds and more importantly from the birds to man (Getz,1980).

Poultry are prone to many parasitic, viral and bacterial diseases. A number of these diseases are prevalent in Zambia all through the year but during the rainy season, climatic conditions become favourable particularly for parasite infestation.

The following are some common diseases in rural poultry: Infectious bronchitis, Newcastle, Aspergillosis, Salmonellosis, Coccidiosis, Fowl pox, Aflatoxicosis and external parasites
(ectoparasites) (Dr. Voetsen, lecture on Poultry Diseases, UNZA, 1993).

1.2.3. THE ANATOMY AND PHYSIOLOGY OF CHICKENS AND DOMESTIC BIRDS.

For descriptive purposes, the chicken (or any bird) can be divided into the exterior (distinguishing it from mammals) and interior (the locomotive, digestive, respiratory, urinary and genital apparatuses) anatomical details.

1.2.3.1. LOCOMOTIVE APPARATUS.

This apparatus consists of bones linked together by joints and set in motion by muscular action. The bones as a whole form the skeleton. Birds also posses a plumage which conditions their flight.

1.2.3.2. RESPIRATORY APPARATUS.

The respiratory apparatus of birds is the nostrils, which open through the beak, passing through the pharynx and the trachea.

1.2.3.3. DIGESTIVE APPARATUS.

This comprises the following organs; mouth, oesophagus, stomach (gizzard), intestine, cloaca and anus, to which are appended the liver and pancreas.

1.2.3.3.a. **Beak:** the beak consists of the upper and lower beaks. At the end/base of the beak in birds of prey and chickens, there is a smooth and waxy ceroma. This covers the entire beak in water birds (goose, duck). As an all-purpose tool of the bird, the beak is used in drinking, eating and scratching and a number of other activities (Koch and Rossa, 1973).

1.2.3.3.b. **Tongue.**
The shape of the tongue corresponds to the form of the mouth cavity caused by the beak form (King and McLelland, 1984). For example, in the chicken and pigeon, the tongue is narrow and pointed;in the goose it is broader and rounded in front. King and McLelland (1984) describe the enormous variety of dietary adaptations displayed in the tongue of birds. Basically, there are three forms of adaptations for collecting foods (long, narrow, protrusible tongue) and adaptations for swallowing (non-protrusible).

1.2.3.3.c. **Oesophagus and Crop.**
After the oesophagus has formed the crop, it enters the stomach. The crop is absent in the purely insect and fruit eating birds, but can serve as food storage in grass-eating birds, especially in chickens and pigeons.

1.2.3.3.d. **Stomach.**
The stomach consists of the glandular stomach, joining the oesophagus, and the muscular stomach that follows it. The glandular stomach produces pepsin-hydrochloric acid. Chickens belong to the group of birds with glandular and muscular stomachs clearly separated from one another (Koch and Rossa,1973). The muscular stomach is a chewing stomach and grinds food that was softened in the crop and mixed with digestive juice in the glandular stomach, by means of small, sharp foreign bodies (stones) taken up with food.

1.2.3.3.e. Intestine.

The intestine generally represents a formation of coils closely wound into each other lying in the body cavity. As in domestic mammals, the duodenum, jejunum, ileum, colon and rectum are found. The total length of the intestinal canal in a chicken is between 165 and 260 centimetres (Koch and Rossa, 1973), measured from beak tip to the pygostyle.

1.2.3.4. PHYSIOLOGY OF DIGESTION.

The search for and recognition of food takes place almost exclusively by means of eyes. The sense of taste seems to participate in the recognition of food in birds that have taste buds and this includes chickens and pigeons. Swallowing is not a peristaltic act because of the absence of a constrictor muscle in the pharynx. There is a passive swallowing in which, through lifting the head, gravity and a reduced pressure in the oesophagus are utilised. However, in the oesophagus the food is seized with peristaltic contractions and conducted toward the crop or stomach. Only after filling the stomach is swallowed food deposited in the crop for storage. While the food is in the crop there is a softening and swelling of the grain food by means of the beak saliva that accompanies it. After passing the crop, the swollen and softened food reaches the glandular stomach and then the small intestine where the main breakdown of the food material takes place after it has been mixed with the pancreatic juice and bile.

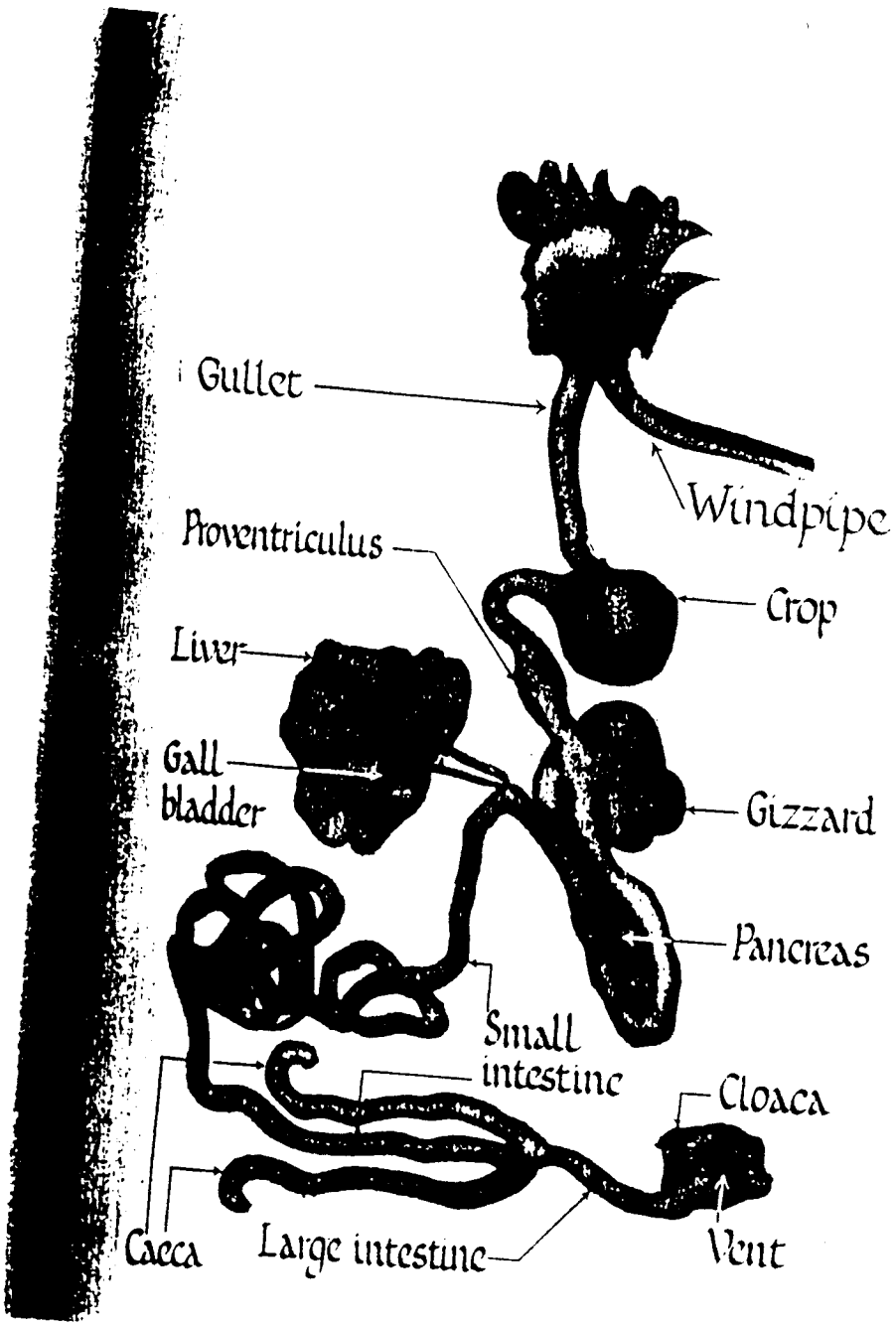
Fig.3

EXTERNAL ANATOMY OF CHICKENS

Cock resting in shade of maize storage bin.

Fig. 4a

DIGESTIVE APPARATUS OF CHICKENS



King and McLelland (1984).

1.2.3.5. URINARY AND GENITAL APPARATUS

The urinary apparatus is identical in the male and female (Say 1984) and consists of the kidney and the ureter. From each of the kidney lobes, small ducts leave, uniting to form the two ureters (one per kidney) which carry the urine to the cloaca. In the cloaca, absorption of water takes place and the urine is excreted as chalk-white pulp due to the uric acid crystals (Koch and Rossa, 1973).

Genital organs (testes in males, ovaries in females) increase at breeding time. In geese and ducks (males), there is a long, curved, fibrous body which represents a copulating organ which can convey semen into the cloaca of the female bird. This is absent in the male chicken.

1.2.3.6. PHYSIOLOGY OF REPRODUCTION.

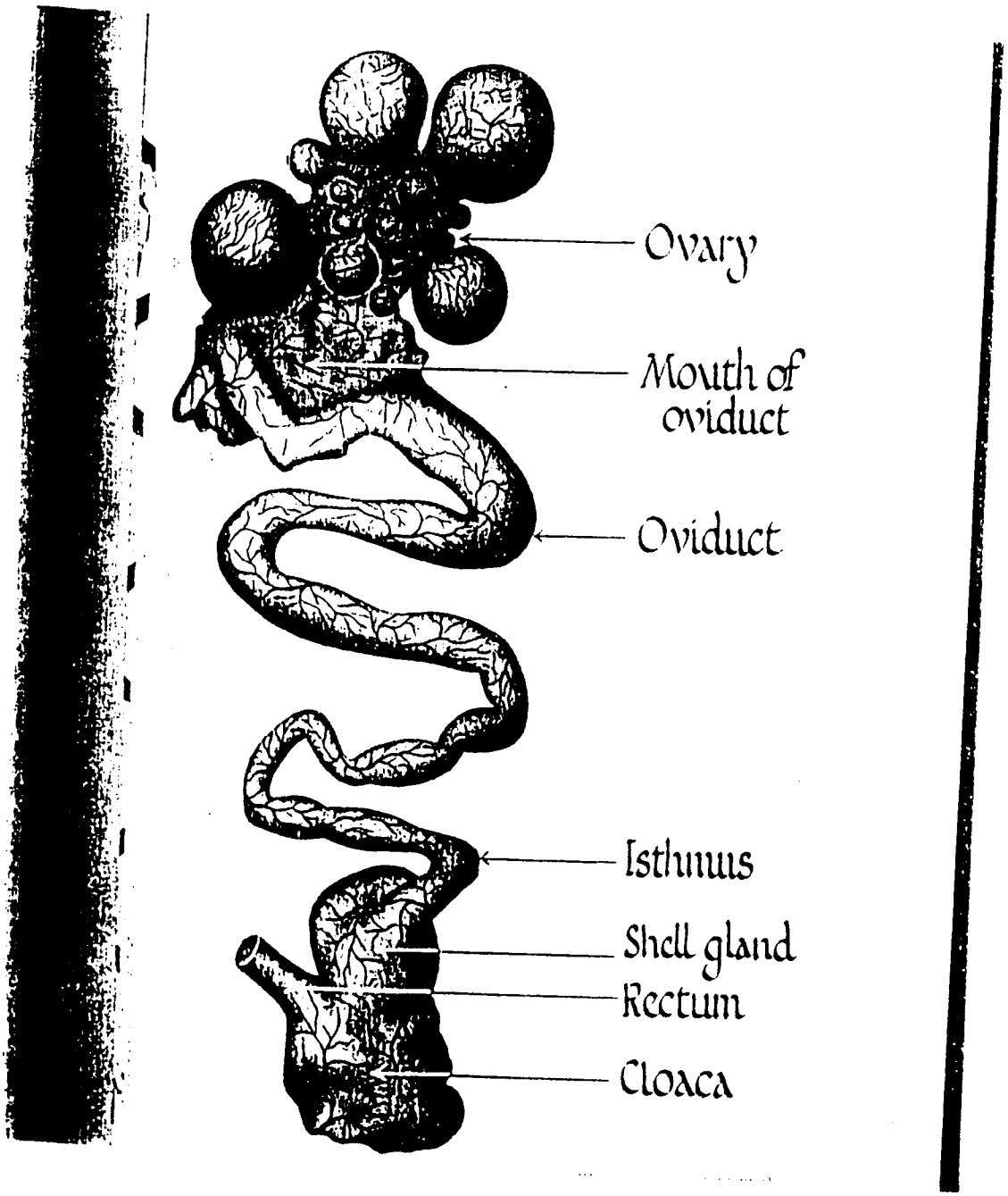
In the male, spermiogenesis takes place when the cock(rooster) is sexually mature at 5 to 7 months of age (Koch and Rossa, 1973). The leybig intermediary cells produce the male sex hormones. In chickens, the estrus periods are curtailed and mating takes place daily. Mating itself takes place by pressing together the cloacae from both partners. After mating, the spermatozoa retain their viability up to 5-6 days.

The ovaries of the female bird are arranged on both sides of the embryo receptacle. In the chicken, only the left ovary with its oviduct is developed. At the age of 4-6 months, the ovary takes up its function. Before ovulation, there takes place within the egg cell maturing divisions. Ovulation itself consists of the tearing of the follicle membrane and the expulsion of the yolk-rich egg cell. Fertilization takes place in the oviduct.

The egg remains in the container for six hours. The passage of the egg (or total time in the oviduct)lasts between 9-22 hours in the chicken (Koch and Rossa, 1973). The laying of the egg occurs by the contraction of the musculature of the uterus (oviduct) as the egg passes out of the vagina. The hormone prolactin, produced by the hypophysis, releases the brood drive which leads to a brooding period lasting up to 21 days in the chicken, after which period the chicks hatch. After hatching, the chicks are immediately sensitive to heatloss and require a brooding temperature of about 32 degrees celsius. Grown chicks require an optimum temperature of 21 degrees celsius with air humidity of 65-70 percent.

FIG. 4b

REPRODUCTIVE APPARATUS OF CHICKEN.



King and McLelland (1984)

1.3. GRAIN FOOD LOSSES IN ZAMBIA.

Zambia has been facing acute food shortages caused mainly by a number of droughts that have occured in the main agricultural provinces in the past. This means that Zambia has had to use scarce foreign exchange to import grain from some neighbouring and far away countries. Other factors and problems, however, also cause the loss of grain food in Zambia and these include lack of adequate and sound storage facilities, lack of transport to move produce to safe storage, poor agricultural policy and unfavourable returns for the farmers, who are predominantly peasant (small-scale) in nature.

As the sizes of the human, animal and poultry populations increase, their impact upon their surroundings also increase as they compete for food. No food plants have been as important in the development of agriculture as the cereals (Barrass, 1974), which provide the year-long food supply that has become the basis for the settled communities. Cereal grains will keep for a long number of years without deteriorating if properly harvested, handled, transported and stored. This means that the grain can be accumulated in years and areas of plenty for use in years and areas of deficiency when crops fail to grow.

Throughout the ages, man has had to struggle to store enough food to live from one season to the next. Man is also in competition for food with animals ranging from monkeys and rodents to bacteria and moulds. Man's ability to store his food successfully and to provide himself with a constant source and a good variety of clean foods has influenced his ability to survive (Kaunda, 1980).

The problem of adequate and sound storage of food remains with Zambia today. It has been argued that infact by storing large amounts of food, man has encouraged rapid increase in the pests that compete with him for the stored food. Food losses in the field and in storage on the world scale were estimated between 10 and 20 percent of the total food produced on the average (Kaunda, 1980). This was, however, higher for Zambia as surveys carried out by the Food Storage and Conservation Unit (F.S.C.U.) in Zambia showed that 30 percent (Kaunda 1980) of the grain stored was lost to pests from one harvest to the next. Losses are more serious in tropical countries as high temperatures and humidities encourage the multiplication of the pests and moulds. In Zambia, the problem of adequate storage is critical in the rural areas where about 33 percent of all the stored food is lost.

1.3.1. FACTORS INFLUENCING GRAIN FOOD LOSSES.

Grain losses have resulted from food being eaten by animals, rodents and insects, soaking by rain, high temperature and high humidity, poor handling and contamination and disease. Some of these factors are dealt with below:

1.3.1.1. BIOLOGICAL AGENTS.

(a) Rodents such as rats and mice. These do not only consume the food but also contaminate it through their droppings, excreta and dropping hairs. They also spread disease to humans. Rodents attack grain both in the field and in store. Their damage is the easiest kind of damage to see.

(b). **Insects** - these attack growing crops and in storage. Losses due to insects are enormous in stored foods where they attack embryos (germs) of grain, which are the most nutritious parts as they contain most of the protein, fat and vitamins of the grain. By so doing, insects reduce the nutritional value of these foods as well as leaving them unfit for sowing. About 72 insect types have been identified that attack grain in Zambia (Kaunda, 1980; Mwiinga, 1985).

The main primary pest attacking undamaged maize in the field and in store are the grain moth (Sitotroga Cerealella) (Olive), and the maize weevil (Sitophilus zeamais Mot). The flour beetle (Tribolium Castaneum) (Herbst) commonly attacks damaged grain in store (Adams and Harman, 1977).

(c) **Microorganisms** - Those most involved in grain spoilage are fungi which are generally found on the surface of undamaged grain. They grow well under certain physical conditions.

1.3.1.2. PHYSICAL FACTORS.

(a). **Temperature** - it is easier to store grain when the temperature is low. In very cold weather, insects and moulds do not grow very quickly, or at all. In warm places, insects start to grow and breed, moulds multiply (Lindblad and Druben, 1974) and cause storage problems.

(b). **Moisture**: This is essential for the growth of storage insects and moulds. An increase in the moisture content of the grain promotes the increase in the storage insects and moulds. Drying grain and keeping it dry in storage is the most important part of good storage. In warm and moist grains, moulds grow and sometimes produce mycotoxins.

(c). **Contamination**: Good hygiene plays an essential role in the adequate storage of grain as hygienic conditions restrict the growth of pests and moulds. It is important to clean the storage place thoroughly before putting new grain in. Damaged storage places should be repaired and cleaned before re-use.

(d) **Handling**: Good grain storage requires careful handling of the grain. Poor handling during harvesting and threshing, transportation and storage causes breakages and damages to the grain. Broken and damaged grain is more susceptible to attack by pests, microorganisms and other insects.

1.3.1.3. LOGISTICAL PROBLEMS.

Much of the losses of maize in Zambia are due to soaking by rains. Every year during the rainy season, which comes 4 months after the harvest season, the harvest is threatened with destruction by rains. For a long time, harvested crops have been soaked by the early rains in rural areas because of lack of storage facilities. Thousands of 90kg bags of maize are soaked and destroyed by the rains every year.

Because of lack of storage facilities, tarpaulins have been used to cover harvested and bagged maize but these are often of poor quality. Sometimes, lack of transport has meant the use of these tarpaulins to cover bagged and stacked up maize in the rural areas. At one rural depot, 80,000 x 90 kg bags (720 tonnes) of maize were reported destroyed in one year because the poor quality tarpaulins covering them allowed water to seep through.

Tarpaulins are also used to cover maize bags in transit from rural depots to "safe" storage mainly along the line of rail. The transport situation in the rainy season, and immediately after, is chaotic. In many rural areas, roads may become impassable. Every year, a serious transport crisis for haulage threatens millions of bags of maize and other crops.

Other logistical problems like lack of spare parts for lorries, suitable markets, shortage of fuel, insufficient empty grain bags and shortage of tyres also cause losses of harvested maize. Most of these logistical problems are associated with a food-path. Food goes along food-paths from the fields where it is grown to the consumer who eats it. Much longer food-paths cause food losses. Foods from outside the country have even longer food-paths indeed as they have to come across the sea/ocean by ship.

Loss in the yield of maize is not due to fungal parasites alone that cause disease in standing crops but a considerable amount of the crop is lost every year by fungi that infect stored maize. The existence of internally carried fungi was shown in Zambian maize (Kapooria et.al,1981) which contained infections caused by one or more species of fungi. Maize appearing apparently healthy may contain up to 80 percent or more infection (Kapooria et.al, 1981). This maize can result in both poor quality and mycotoxin contamination of the grain. Fusarium moniliforme, Aspergillus, flavus and other fungi are known to produce toxins.

1.4. MAIZE STORAGE METHODS IN THE RURAL AREAS.

In the rural areas of Zambia, maize and groundnuts are grown mainly to provide food for the families and only the surplus is sold immediately after harvest. Since maize and groundnuts, which provide a big proportion of the diets, are high-aflatoxin risk substrates, they need some sound storage facilities. Mycotoxin occurrence is of major concern in rural area situations where the conditions of grain storage can lead to spoilage and wastage of grains.

The main role of storage in the village is to overcome the problem of seasonality, and maintaining the supply of the commodities between one harvest and the next. The dry maize is stored in traditional and very ingenious receptacles constructed from local grasses and trees. These are cheap and less secure. Ideally, people in rural areas store cobs with or without husks, as well as shelled grain. The stores built by the Tonga people of Southern Province traditionally hold cobs and have conical grass-thatched roofs. There are two traditional stores, both consisting of a cylindrical "basket" on a raised platform, which is normally made of branches supported by strong Y posts. The basket itself may be made on branches placed on vertical poles (Fig.5.a.), or it may be a "woven" cylinder of intertwined twigs (Fig.5.b.).

Both types of stores usually have an aperture cut in the top of the wall for access to the cobs, enabling it to be emptied with the roof in place(Adams and Harman, 1977).

In Chalimbana, Lusaka Rural, maize is stored by the Soli after shelling and it is kept in muddied stores with a small access hole near the base (Fig.5.c.).The access hole is closed by a stone or tin which, on removal, allows the grain to pour out into a container (Adams and Harman, 1977).

The grain stocks are normally stored throughout the rainy season when mould growth can occur readily due to favourable temperatures and humidity. In addition, the large sizes of most families in the rural areas require that a lot of grain is stored and this is packed so tightly that not enough air is allowed to circulate causing the accumulation of moisture by condensation. This is worse if the grain was harvested when it was not completely mature and dry.

Much of the losses of food in storage in the rural areas, with all its tragic consequences, could be prevented and reduced if only families would make stronger storage bins by improving on those that are available. This is important because a weak storage bin cannot stand the heavy rains that fall sometimes. A strong bin, on the other hand, is not harmed by rains and ensures safety of purposely stored maize. Regular maintenance consisting of re-roofing, cleaning out thoroughly and replacement of broken termite infected poles is required.

The valuation of losses in stored grain may be based on its intended use. Maize is stored predominantly for food but may be sold, bartered, fed to animals and poultry, and serve as seed for sowing the next year's crop. Usually only rotten maize is fed to poultry as damaged maize is rarely thrown away. However, damaged maize, and sometimes rotten maize, is used for making into beer.

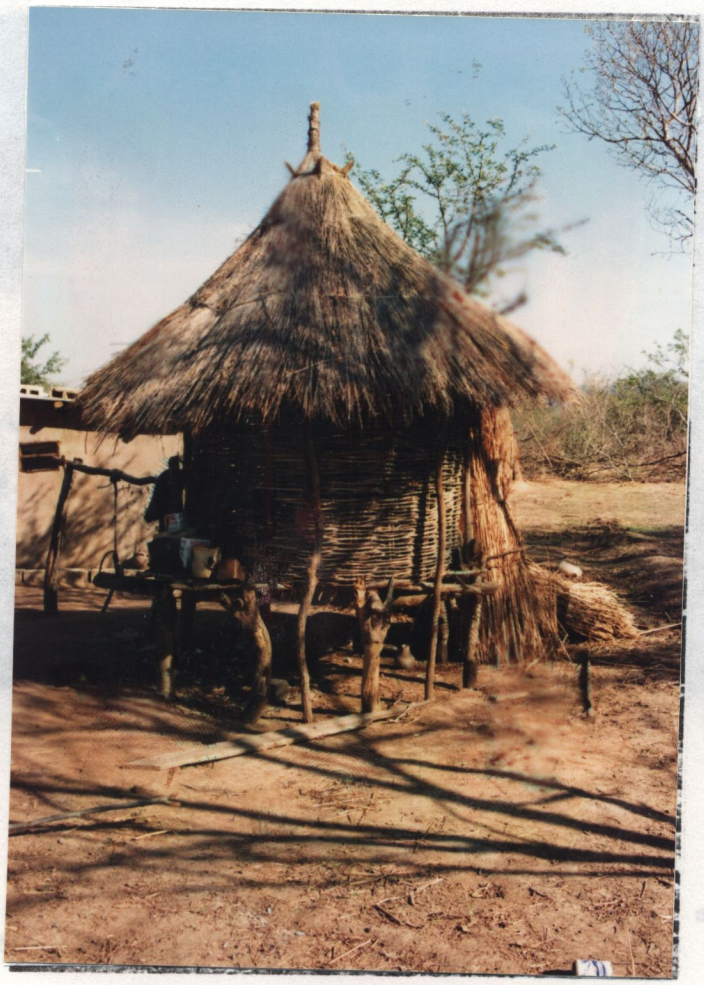
Fig. 5a

TRADITIONAL MAIZE STORAGE

(a) POLE TYPE: basket made of vertical poles.
Picture: Kafue, Southern Province, Zambia.

Fig. 5b

TRADITIONAL MAIZE STORAGE



(b) BASKET TYPE: basket made of woven cylinders of intertwined twigs.
Picture: Kafue, Southern Province, Zambia.

Fig. 5c

TRADITIONAL MAIZE STORAGE

(c) MUDDIED TYPE: pole or basket type smeared with mud to seal holes.
Picture: Choma, Southern Province, Zambia.

1.5. AFLATOXINS AND OTHER MYCOTOXINS.

1.5.1. HISTORY AND BACKGROUND TO THE DISCOVERY OF MYCOTOXINS.

Mycotoxins have been described by Mannon and Johnson (1985) as a mixed bag of poisonous and often cancer-causing chemicals produced by a variety of moulds that can grow on foods, particularly on grains and nuts. Hundreds of species of moulds that grow on foods secrete one or more mycotoxins. Most mycotoxins present in a wide range of agricultural commodities are, however, usually present in insignificant quantities in nature (Robb, 1993).

The moulds are a diverse group of eukaryotic, heterotrophic organisms, the latter distinguishing them from plants (Samuel, 1987). The moulds are the area of interest in this study. Many of the moulds produce a diverse range of secondary metabolites, some toxic(for example aflatoxin) and others non-toxic and beneficial (e.g. vitamins and antibiotics). Among the toxic metabolites are the mycotoxins which when ingested by man, poultry and animals may cause disease. Of the many species of fungi which have the ability to produce mycotoxins, those belonging to the genera Aspergillus, Fusarium and Penicillium are considered among the most important (Robb, 1993).

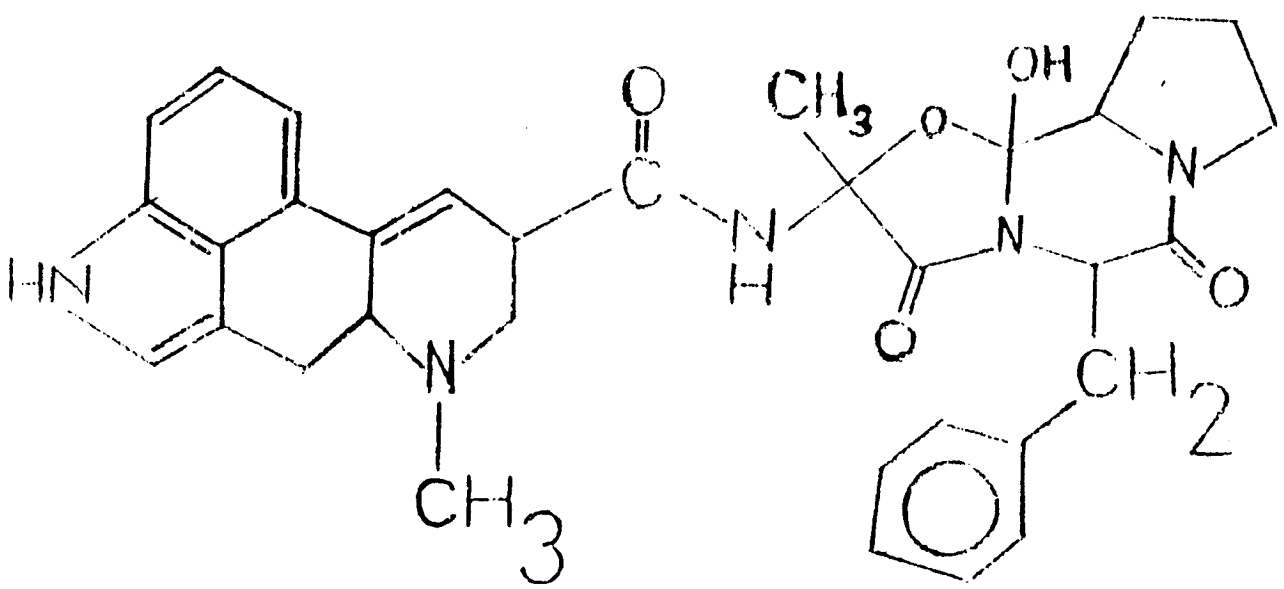
It should not be thought that fungi are predominantly harmful. Generally, they are more useful and beneficial to man and animals. While it is true that moulds are involved in the spoilage of many kinds of foods, some moulds are useful in the manufacture of certain foods or ingredients of foods e.g. as in single cell protein. A beneficial, non-toxic fungus, Scropulariopsis, overgrows most other fungi thereby controlling them and decreasing incidence of disease produced by them . Other fungi such as Penicillium and those involved in the production of antibiotics (active against bacterial growth) and vitamins are positively valuable (Hungerford, 1970).

To emphasize how diverse a range of fungi may be implicated as aetiological agents,it is worth recalling two historic examples- ergotism caused by Claviceps purpurea (or ergot) and facial eczema of sheep caused by Pithomyces chartanum. Mycotoxicoses or mouldy feed poisoning tend to be given special names such as aflatoxicosis(produced by Aspergillus flavus) starchy botriotoxicosis (caused by Starchybotrys alternams) and so on.

1.5.1.1. ERGOTISM.

The most familiar and the oldest recorded example of these mycotoxins is ergotin, a powerful toxin found on grains (cereals) and other grasses, particularly rye. The first mention of ergot dates from 600 BC. Epidemics of ergotism were often preceded by periods of hunger and famine which presumably necessitated the consumption of contaminated grain that contained ergotamine (fig.6).

Fig. 6
ERGOTAMINE



Mycotoxin found on grains causing ergotism.

A chronicler of this time described what happened in the epidemic of 940 A.D. Wailing and writhing men collapsed on the street; others fell over and foamed in epileptic fits while some vomited and showed symptoms of insanity. Many of them shouted "Fire! I'm burning". The chronicler wrote: "It was an invisible fire that separated the flesh from the bones and consumed it. Men, women and children died in unbearable pain. Firstly, their toes turned black, then their fingers burst open, their legs and arms convulsed and fell off. A terrible, painful roar could be heard miles away and the horrible smell remained in the streets for weeks" (Wyllie and Morehause, 1980).

Two distinct forms of ergotism, a convulsive form and a gangrenous form could occur. Gangrenous ergotism was recorded in France where it came to be known as St. Anthony's fire. The symptoms were feeling of cold hands and feet, and a horrible burning sensation which were followed by limbs turning black and finally falling off. In Russia and eastern Europe, a convulsive form was common and symptoms were accompanied by fits, hallucinations, blindness, abortion and loss of blood, with death coming rapidly (Wyllie and Morehause, 1980).

The different symptoms were called St. Anthony's fire or St. Vitus's dance because victims visited the shrines of St. Anthony and St.Vitus in the hope of being cured. Thousands of people died during the outbreaks but the involvement and presence of the ergot mould and the toxic substances were only identified in the early 1900's (Purchase, 1973). Until the people knew their sufferings, they had no rational method of treatment. Christensen (1975) reports that when epidemics of ergot poisoning scourged the human populations, as described above, some ergot poisoning probably occurred in domestic animals and poultry too, although there are no records of this (Wyllie and Morehause, 1980).

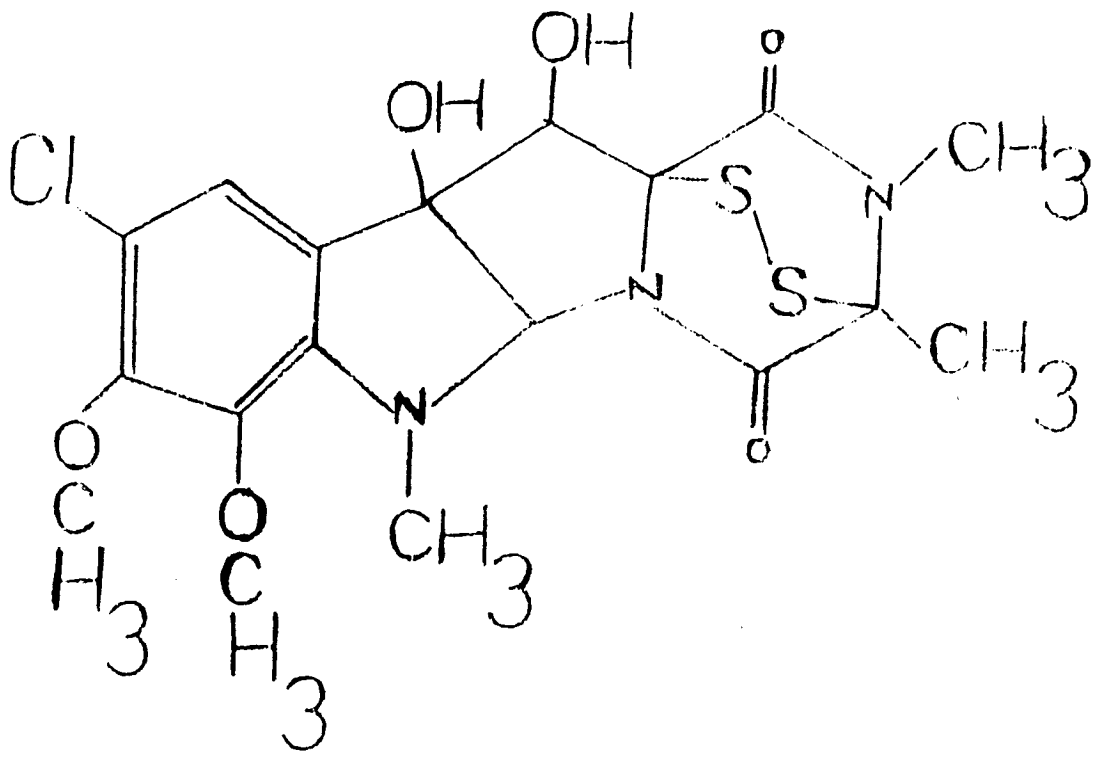
1.5.1.2. FACIAL ECZEMA.

Facial eczema was usually thought of as a disease of ruminants (sheep) but it occurs also in cattle though the bovine species is rather less susceptible to the facial eczema toxin (Dodd, 1964). Subsequent to the outbreak, several features of the disease were recognised and formed the basis for future research. Common features of the outbreaks were that they occurred from January to May (during warm, sunny weather) and sheep and cattle of all ages could be affected. The fungus that was eventually incriminated as the cause of facial eczema was Pithomyces chartanum and its toxic metabolic product is "sporidesmin". This name was chosen for the toxin when the fungus was known as Sporidesmin bakeri (Dodd, 1964) (Fig. 7) This fungus has a worldwide distribution.

It is difficult to be precise about the groups of mycotoxicoses (illnesses) of man and his domesticated animals because toxic fungal metabolites are chemically diverse (U.S. Task Force Report, 1989).

Fig. 7

SPORIDESMIN A



Mycotoxin causing facial eczema in ruminants.

1.5.1.3. ASPERGILLUS.

There are several ways in which Aspergillus can cause disease in man (Moss, 1977). Disease may be produced by one of the following roles of the organism (Moreau, 1979): as an infective agent, as a saprophyte, by producing allergic reactions and mycotoxins.

1.5.1.4. DISCOVERY OF MYCOTOXINS.

The study of mycotoxins began with the discovery of aflatoxins. The toxin discovered was named aflatoxin, the "a" from Aspergillus and the "fla" from flavus. The history of the discovery of what are known as mycotoxins (and their resultant mycotoxicoses) stretches as far back as the early 1960's when a British scientist Blount (1961)first reported and described the problem in animal and poultry health. The problem of mycotoxins including aflatoxins, other aspergillotoxins, clavacitoxins, fusariotoxins and trichothecenes came to the attention of scientists, veterinarians, biochemists, nutritionists etc. because, of an apparently new and mysterious Turkey "X" disease (Campbell, 1977). This was named as such because a lot more turkeys than other species died.

During the summer and spring of 1960, numerous outbreaks of a disease of an unusual nature occurred (Asplin and Carnaghan, 1961) in turkey poults aged between 3 and 20 weeks fed on proprietary and home-compounded rations. Losses were heaviest in poults between 3 and 6 weeks of age and mortality extended up to one hundred percent in some affected groups(Sargeant et,al, 1961).

Most of the outbreaks of the disease occurred in the South and East of England. In all, some 500 outbreaks of Turkey "X" disease were diagonised and it was estimated that more than 100,000 turkeys died (Blount, 1961). Ducklings and young pheasants were also affected with a similar disease and heavy mortality was experienced by many rearers. Chickens fed diets similar to those which affected turkeys, ducklings and pheasants, showed retarded growth but low mortality (Sargeant et.al, 1961)

The outbreak of the disease received considerable publicity in the local press. Asplin and Carnaghan (1961) got the report in August 1960 that over 14,000 ducklings had died on one farm alone during a 5-week period. On this farm in East Anglia, only home-compounded ration had been fed, the protein-rich ingredients being the Brazilian groundnut meal and fish meal (Sargeant et.at., 1961).

During the same time, reports were received from Kenya and Uganda of severe losses in ducklings (Asplin and Carnaghan, 1961). The description of the condition was similar to that seen in England in ducklings fed on diets containing Brazilian groundnut meal. On enquiry, it was learnt that the Brazilian groundnut ingredient was not present in the rations with which the disease was associated but that they did contain some groundnut meal from groundnut which had been grown in Uganda and Tanzania. When this groundnut meal was withdrawn from the rations, losses ceased. In the United Stated of America, an outbreak of trout hepatoma was discovered. Pigs and calves seemed to be affected by a similar disease (U.S. Task Force, 1989).

Following the Turkey "X" disease outbreaks with the deaths and economic losses suffered by the poultry rearers, some investigations were instituted in search of the causative agent(s) (Blount, 1961). The fact that when the groundnut meal ingredients were withdrawn from the rations, the losses reduced or ceased seemed to indicate that the disease in ducklings, poults and pheasants had a common cause (Asplin and Carnaghan, 1961). This was traced to be due to a dietectic factor as a change of food frequently resulted in improvement (Blount, 1961). Blount (1961) also examined the incidence of the disease in turkey poults in a number of areas in the United Kingdom and showed the common factor to be the imported Brazilian groundnut meal in the rations which had been imported in the previous year.

Since the groundnut meal in question had been in use for several years and was known to provide an excellent source of protein for poultry and animals, it was unlikely that the feed itself was toxic. It was, therefore, reasoned rightly that some toxic ingredient could have been added in some way by some toxin-producing fungus growing in the feed (Christensen, 1975).

The fungus Aspergillus flavus was isolated from the Brazilian meal, identified and characterised as the causative agent after it was grown in pure culture and fed to turkey poults. It killed them, with external signs and internal lesions identical to those detected in the birds that had died in the field (Christensen, 1975). It has since been established that many toxic moulds grow and proliferate on a variety of foods, their toxins contaminating the foods so that the consumer (humans, animals and/or poultry) suffers the results of contamination (Hesseltine and Mehlman, 1977).

Further investigations soon showed that the causative agents (the mycotoxins) were actually secondary metabolites of the isolated fungus (Newberne and Butler, 1969). Allcroft and Carnaghan (1960) prepared a toxic extract, from the Brazilian groundnut meal samples, which proved to be highly toxic to young ducklings. Sargeant et,al,(1961) also prepared similar extracts from certain samples of East African groundnut meal by a development of the method of Allcroft and Carnaghan (1960). In feeding trials using these extracts and ducklings, there was no doubt that the condition produced by these East African samples was identical to that caused by toxic samples from Brazilian groundnut meals.

Sargeant et.al (1961) reported that they analyzed various other groundnut samples from India, Nigeria, Gambia and Ghana and found a toxic factor similar to that in Brazilian groundnut meal. It was a belief by then that the groundnuts were the responsible factor. Contrary to this, in the United States, the cottonseed component in the ration was found to be the toxic agent (Wolf and Jackson, 1963). This finding soon showed that the toxic factor was not confined to just the groundnut meal.

According to Goldblatt (1969), Turkey "X" disease was characterised by anorexia and resulting retardation of growth,lethargy and weakness of wings. The turkeys soon developed a staggering gait before they collapsed and died. A characteristic position was assumed at death with the head drawn back on an arched neck and the legs extended fully backwards. Postmortems on the affected birds revealed pathological changes in the livers.

These were summarised by Newberne and Butler (1969) as "severe periportal hepatic parenchymal cell necrosis and venous congestion and regeneration accompanied by the diffuse necrosis of cells and concomitant biliary proliferation" for all species after continuous administration of aflatoxin.

In young poults fed toxic feed from the age of one day, symptoms may take up to 2 weeks to develop. Lethargy and loss of appetite, drooping wings and broken feathers in the flock are followed by deaths, often characterised by spasm of the neck muscles and death with the legs stretched posteriorly in full extension. The main lesions occur in the liver, which may be yellow-brown in colour or mottled. In some birds which recovered, paleness and hardness develops into full cirrhosis.

1.5.1.5. CHEMICAL STRUCTURE OF AFLATOXINS.

The toxic factor isolated from cultures of A.flavus and responsible for the Turkey "X" disease was soon identified. At the time it was not known that this toxic factor was not just a single entity but a mixture of several structurally similar components. Neither was it realised that the composition of mixture depended on the particular strain of A. flavus as well as the prevailing growth conditions.

The aflatoxins are a group of bisfuranocoumarin metabolites isolated from strains of A. flavus and A. parasiticus groups of fungi. A single toxic factor, as it was initially thought to be turned out later to be a complex mixture of various metabolites of different toxicities. There were 4 primary aflatoxins, later named B1, B2, G1 and G2 (Christensen, 1975), with aflatoxin B1 being the most toxic of the group.

Sargeant et.al.(1961) demonstrated that the toxic principle in the Brazilian groundnut meal could be extracted and resolved by paper chromatography. When the "purified" extract was resolved, a single spot which exhibited a bright blue fluorescence under ultraviolet light (wavelength 365nm) was detected. It was soon showed that this single blue-fluorescence spot could be further resolved into two spots, of different retardation factor (Rf). One spot had a blue fluorescence while the other fluoresced green at a slightly lower Rf value. These were given the trivial names aflatoxin B and aflatoxin G, respectively, because of the colour of fluorescence.

In 1963, Hartley et.al. reported that they had isolated and characterised four closely related toxins on silica gel plates using chloroform-methanol (98:2 v/v) as the developing solvent. Two of these toxin spots had Rf values of 0.40 and 0.36, fluoresced blue-violet and were designated aflatoxins B1 and B2, respectively. The other two had Rf values at 0.34 and 0.31, fluoresced green and were designated aflatoxins G1 and G2, respectively.

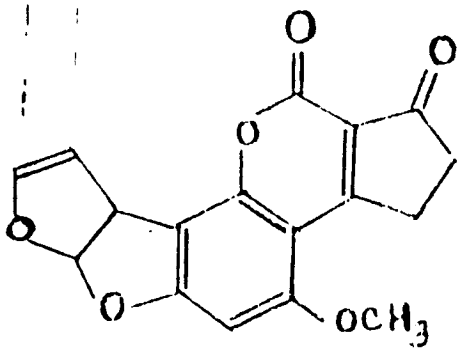
Research continued and this was soon followed by the isolation of numerous other metabolites of the main aflatoxins (Fig.8) It was only after the chemical and structural nature of aflatoxins was determined that it became evident that several instances of aflatoxicoses (disease caused by ingestion of aflatoxins) had been previously observed in both experimental and farm animals and poultry.

1.5.1.6. ESSENTIAL CONDITIONS FOR PRODUCTION OF THE AFLATOXINS.

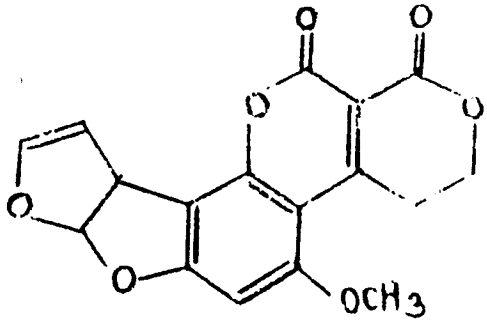
Aspergillus flavus is an ubiquitous mould found throughout the world. However, isolates of A. flavus vary widely in the ability to produce aflatoxins and some strains do not produce aflatoxins at all (Jones, 1975), so that the mould growth does not necessarily indicate the elaboration of aflatoxins. The presence of aflatoxins in a wide variety of agricultural products, including cottonseed, maize, and groundnuts (Njapau personal comm.) demonstrates the lack of a specific aflatoxin-substrate relationship. The conditions for the growth of A. flavus and aflatoxin production on various substrates has been extensively studied. Fortunately, the conditions necessary for A. flavus to produce appreciable amounts of toxin are somewhat narrower and more restricted than the conditions for it to grow (Christensen, 1975). In general, aflatoxin production depends on the moisture, the temperature, the nature of the substrates and the available oxygen (Jones, 1975). These major environmental factors that determine whether it will grow sufficiently to produce toxins are discussed below.

Fig. 8

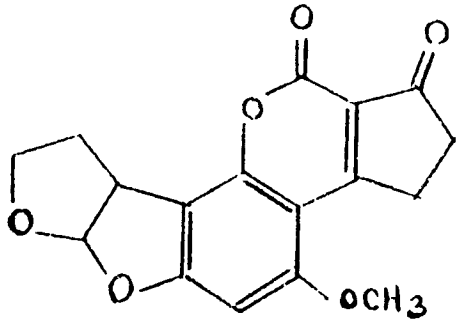
AFLATOXIN STRUCTURES



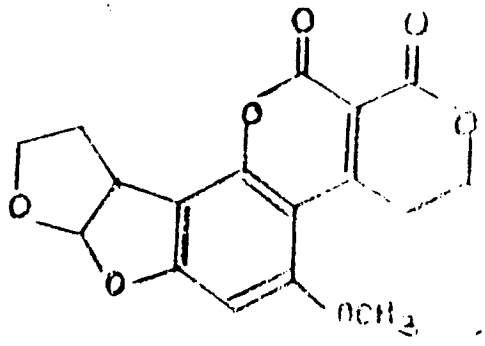
AF B_1



AF G_1



AF B_2



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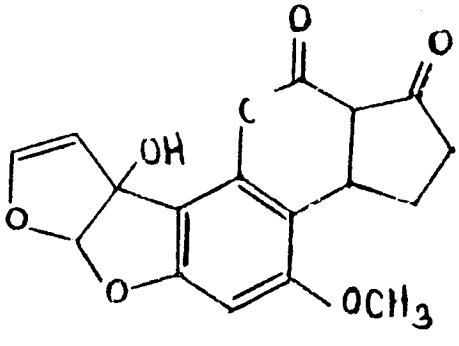
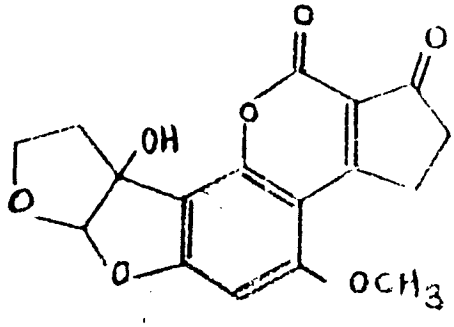
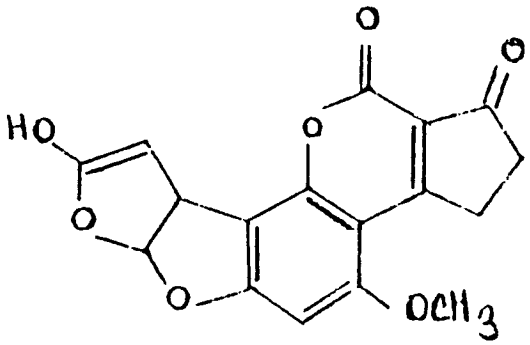


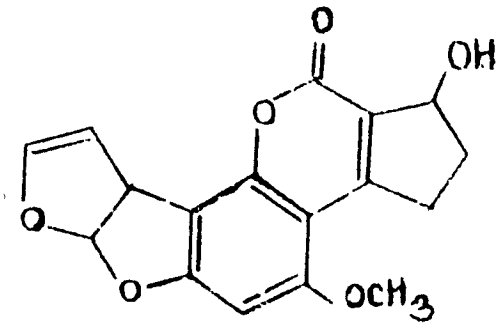
AF M_1



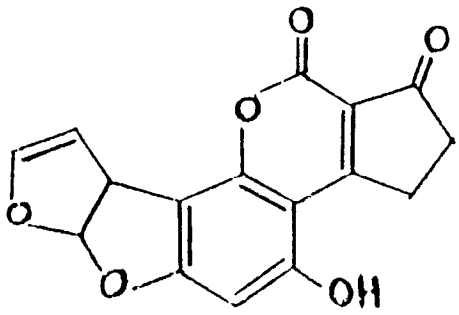
AF M_2



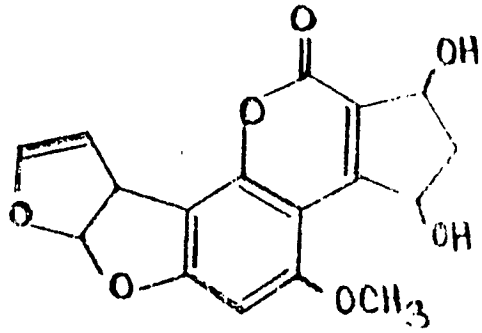
AF B_{2a}



aflatoxicol



AF P_1



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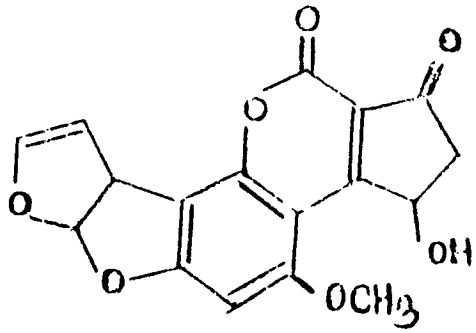


AF Q_1

1.5.1.6.a. Moisture.

One of the most important of these environmental conditions is the moisture content of or the relative humidity surrounding the substrate (Austwick and Aryerst, 1963). The optimum relative humidity for aflatoxin production is about 85 to 90 percent (Diener and Davies, 1967)and the minimum relative humidity for growth of A. flavus is 80 percent (Panassenko, 1941). Unlike some other fungi, A. flavus does not have a maximum moisture content for growth - the higher the moisture content above the maximum (lower limit) the faster the fungus' growth (Christensen, 1975). This means that the minimum substrate moisture content for A. flavus growth and aflatoxin production will depend on the substrate itself (Auswick and Aryerst, 1963). In the starchy cereal grains, such as wheat, corn, rice and sorghum, this is a moisture content of 18.5 to 19.0 percent, wet weight basis. In groundnuts, it is a moisture content of about 8.0 to 9.0 percent. Provided cereal grains and groundnuts are dried to below critical levels and provided that the moisture levels are kept below these levels during storage, the risk of aflatoxin contamination or invasion by A. flavus is minimised (Jones, 1975).

1.5.1.6.b. Temperature.

Like any biological process, the production of aflatoxin is temperature dependent. The minimum temperature for the production of aflatoxin by A. flavus is 12 degrees celsius, the optimum is 27-28 °C, the maximum is 42 °C (Christensen, 1975). The A. flavus fungus will grow slowly below the minimum temperature for the production of aflatoxin, and will grow rapidly at a temperature 5 to 10 degrees above the maximum temperature. The A.flavus fungus at 25 to 33 degrees celsius usually requires 6-8 days for production of large amounts of aflatoxin to commence and the maximum appears within 10 to 13 days (Table 1).

When A.flavus grows as a predominant organism in stored grain it may raise the temperature of the grain in which it is growing to 45 to 50 degrees celsius and hold it there for some time (Christensen, 1975).

1.5.1.6.c. Substrate.

Aflatoxins have been found in many raw and manufactured (processed) products and have been produced in even more kinds of substrates in the laboratory. The species of Aspergillus depends on the nature of the substrate. Given an equal growth of A.flavus, much more aflatoxin will be formed in one substrate than the other. The fungus, for example, grows about equally well in both soyabeans and groundnuts, yet very little aflatoxin is produced in soyabeans, whereas large, sometimes extremely large, amounts may be produced in groundnuts (Christensen, 1975). Thus, some food and food substrates can be rated as of low or moderate aflatoxin risk and others as of high aflatoxin risk.

TABLE 1.

APPROXIMATE MINIMUM OPTIMUM AND MAXIMUM TEMPERATURES FOR GROWTH OF COMMON "STORAGE" FUNGI ON GRAINS.

	MINIMUM (DEG. CELSIUS)	OPTIMUM (DEG. CELSIUS)	MAXIMUM (DEG. CELSIUS)
ASPERGILLUS RESTRICTUS	5 - 10	30 - 35	40 - 45
ASPERGILLUS GLAUCUS GROUP	0 - 5	30 - 35	40 - 45
ASPERGILLUS CANDIDUS	10 - 15	45 - 50	50 - 55
ASPERGILLUS FLAVUS	10 - 15	30 - 35	45 - 55
PENICILLIUM SPP	-5 - 0	20 - 25	35 - 40

WYLLIE AND MOREHOUSE, 1980.

The way foods and/or foodstuffs are handled also affects aflatoxin risk. In some villages in Zambia, it is customary to cook up enough maize or whatever else constitutes the staple diet to last for at least two days. This exposes the food to invasion by fungi. If the fungus is a toxin-producing strain of A. flavus and the weather is right (especially a warm, rainy season), appreciable amounts of aflatoxin may be produced in the food before it is consumed. As Christensen (1975) observes and concludes, "A combination of high-aflatoxin-risk material, high aflatoxin-risk climate and high aflatoxin risk practises in handling and preparing food of course increases the chances of aflatoxin poisoning".

In the case of maize and groundnuts which tend to be highly susceptible to contamination, the main contributing factors are damage to the shell and splitting of the kernels. This can be caused by insects, drought and poor harvesting practices. Freshly harvested groundnuts are generally free from contamination with aflatoxin even when the pods are infected with A.flavus (Jones, 1975).

1.5.1.7. OCCURRENCE OF MYCOTOXIN CONTAMINATION.

Moulds occur everywhere and their spores are present in the air. Climatic conditions in tropical and sub-tropical countries often provide enabling environments that are ideal for rapid mould growth because of the high humidity (during the rainy season and immediately after) and warm temperatures. Moulds can cause contamination and spoilage of both raw and manufactured foods. Serious contamination of harvested "dried" products can occur in villages and rural areas where due to ignorance and unawareness of the basic principles of sound storage, mould colonisation of stored maize occurs. Food may serve as a vehicle for the distribution of two major groups of organisms pathogenic to man: micro-organisms that contaminate food and may cause intoxication in man, and those that infect animals and are transmissible to man (Zoonoses) (World Health Organisation, 1972).

The contamination of foods may take place before the food is harvested, during gathering, or during transportation and storage. The natural occurrence of aflatoxins has been reported in edible nuts (groundnuts, brazil nuts, pistachio nuts), oil seeds (cotton seed, copra) and cereal grains (maize, sorghum, wheat, rice) together with beans (Coker, 1979; Njapau, personal comm.). Since maize and groundnuts are used extensively as both human food and animal feed, the aflatoxin problem associated with them has been extensively studied. Foods processed from groundnuts, maize and milk have received the greatest attention because these are the major raw products that have been surveyed for mycotoxin contamination. Aflatoxin-contaminated maize and cottonseed meal in dairy rations have resulted in aflatoxin M1 -contaminated milk and milk products.

The natural occurrence of mycotoxins and their metabolites in meat products can occur as residues from consumption of the mycotoxins in mouldy feed. Ochratoxin A has been the mycotoxin commonly found as a residue in pork and poultry meat (U.S Task Force Report, Nov 1989) and aflatoxin residues have been demonstrated experimentally in animal and poultry organs, tissues and even eggs by some researchers.

Mycotoxins can be identified as occurring in agricultural products and processed foods. Mycotoxins have been found to contaminate many imported crops as well, sometimes at very high concentrations. In one import into Japan from the United States, popcorn was found to have 84,000 mg/Kg deoxynivalenol (U.S. Task Force, Nov. 1989). The commodities with a high risk of aflatoxin contamination include maize and groundnuts, and to a lesser extent cottonseed. Those with a lower risk of aflatoxin contamination include soya beans, beans, cassava, millet, sorghum and rice. These are resistant or only moderately susceptible to Aspergillus growth and aflatoxin contamination in the field. However, aflatoxins can occur when they are stored under high moisture and temperature conditions i.e. they are potentially susceptible to mycotoxins.

Mould growth on foods, with its fuzzy or cottony appearance, is familiar to everyone. Food with the cottony appearance is considered spoiled and unfit for human consumption and usually rejected. However, this judgement will depend on an individual consumer. Starving people might eat food they would not consume under "normal" conditions.

1.5.1.7.1. FUNGI AFFECTING CEREALS.

In general, the fungi that invade cereal grains and their products may be divided into distinct groups: (1) field fungi, (2) storage fungi and possibly a third group designated advanced decay fungi (Christensen, 1964).

1. **"Field" Fungi**: these invade the grain or kernels while these are developing on plants in the field, or after the seeds have matured but before they are harvested. In wheat and barley, the principle genera of field fungi, in order of predominance, are Alternaria, Fusarium, Helminthosporium and Cladosporium, but other genera are encountered commonly in maize, where they cause or are associated with cob rot. These fungi normally do not continue to grow after harvest, because grains are harvested with a lower moisture content (22-25 percent wet weight) than they require to grow. The field fungi gradually or rapidly die in stored grain (Christensen, 1964).

2. **"Storage" fungi**: these mainly invade grain after harvest and in storage and are adapted to limited water (Christensen, 1964). These include Aspergillus restrictus, Aspergillus glaucus group, Aspergillus candidus, Aspergillus flavus and Penicillium spp. which invade grain stored with moisture contents of 13-18 percent. Some fungi can invade the grain in the field but continue to grow under storage conditions.

3. **"Advanced decay" fungi** resemble field fungi in their moisture content requirements, but do not commonly invade grain extensively before harvest. These include Papulospora, Chaetomium and Sodaria.

1.5.1.8. LEGISLATION AND CONTROL OF AFLATOXINS.

Because mycotoxins are naturally present and unavoidable contaminants in foods and feeds, the exposure of humans (Schuller and van Egmond, 1983), animals and poultry can not be prevented completely. Thus, the exposure of the population to some level of mycotoxins must be accepted.

Aflatoxin is the mycotoxin most widely controlled under statutory laws and regulations. The criteria used in setting these aflatoxin limits in animal feeds are that the allowed level of mycotoxin should not (i) impair the health of the animal or poultry; and (ii) be sufficient to enter the human food via animal and poultry products.

To protect the public health, food laws in most developed countries prohibit the sale of any foodstuff that contains substances (harmful) that may cause injury to animals, poultry and humans. This includes several mycotoxins (Schuller and van Egmond, 1983). In a number of countries, the general legislation has been translated into regulations that impose limits or tolerances on the concentrations of specific mycotoxins. Countries vary in the actual limits which they have set for the maximum permissible aflatoxin concentrations in human foods and poultry and feeds (Tables 2 and 3). These are measurable levels of contact below which there is no significant threat to health. These acceptable levels of contact are called threshold limit values (World Health Organisation, 1972).

Some countries include all foods and feeds in the control (Japan, Poland, Singapore, South Africa, Sweden, U.S.A.) whereas only a few food commodities that are likely to be heavily contaminated are covered in other countries (Canada, Denmark, India, Italy, Malawi, Netherlands). A few countries have only established tolerances for feeds (Belgium, France, Israel) and some countries have established tolerances for export commodities only in particular Brazil (Krogh, 1977) and Zambia (Samuel, 1987; personal comm. from the Food and Drug Unit in Zambia). Zambia has a 10 ug/kg Aflatoxin tolerance level for groundnuts meant for export (Kannaiyan et.al., 1985).

Some countries (e.g France) allow the importation of feed commodities containing aflatoxin B1 with concentration much higher than the threshold limit values on condition that they go straight to feed manufacturers who will incorporate feedstuffs into animal/poultry feeds at an inclusion rate that does not exceed the lawful limit. This compromise is not allowed for countries with a zero limit for all foods and feed.

Table 2

MAXIMUM PERMITTED LEVELS FOR AFLATOXIN IN FOODSTUFFS

Country (a)	level (ug/kg)	Product(s)
Australia (Victoria state only)	15 $AFB_{1)}$ 5 AFB_1	Groundnuts and Groundnut products other foods
Canada	15 (total aflatoxins)	Edible Nuts and Nut Products
Denmark	10 (total aflatoxins)	Groundnuts and Groundnut Products
India	60 AFB_1	Groundnut Flour
Italy	50 AFB_1	Groundnuts and Groundnut Products
Japan	0 (detection limit of method 10 ug/kg $AFB_{1)}$	All Foods
Netherlands	0 (detection limit of method 5 ug/kg	Groundnuts and Groundnut Products
Poland	0	All Foods
Singapore	0	All Foods
South Africa	10 (total aflatoxins) max. 5 aflatoxin B_1	All Foods
Sweden	5 (total aflatoxins)	All Foods
Switzerland	6 (total aflatoxins) 0.05 aflatoxin M_1	Roasted Peanuts Milk, specified Milk Products
UK	30 (total aflatoxins)	Nut and Nut Products Imports
USA	25 (total aflatoxins) 20 (total aflatoxins)(b)	Raw Unprocessed Groundnuts Edible Nuts and Nut Products
West Germany	10 (total aflatoxins max. 5 aflatoxin B_1	Edible Nuts and Nut Products

Wyllie and Morehouse, 1980

Table 3: AFLATOXIN LIMITS FOR FEED IN VARIOUS COUNTRIES

COUNTRY	TYPE OF CONTROL	COMMODITY	AFLATOXIN LIMIT (µg/kg)	REMARK
Brazil	Govt. Decree	Groundnut meal	50	Export control
EEC	Council Directive	All mixed feeds & daily supplement	10 - 50	Dependant on animal
France	Regulation	Groundnut meal	700	Import control
Japan	Regulation	Groundnut meal	1000	0% Inclusion for: chicks, calves & pigs. 3% dairy, cattle, 5% other livestock
Norway	Regulation (Min.of Agric.)	Oilseed meals	600	8% in feed concentrates
Sweden	Advisory Standard	Groundnut meal	600	Dairy feed 15%
UK	Regulation (Min. of Agric.)	Groundnut meal	50	Import control
Zimbabwe	Voluntary Code	Mixed feeds	50 - 400	Dependant on animal

Wyllie and Morehouse, 1980

1.5.1.9. HUMAN EXPOSURE TO AFLATOXINS (AND OTHER MYCOTOXINS)

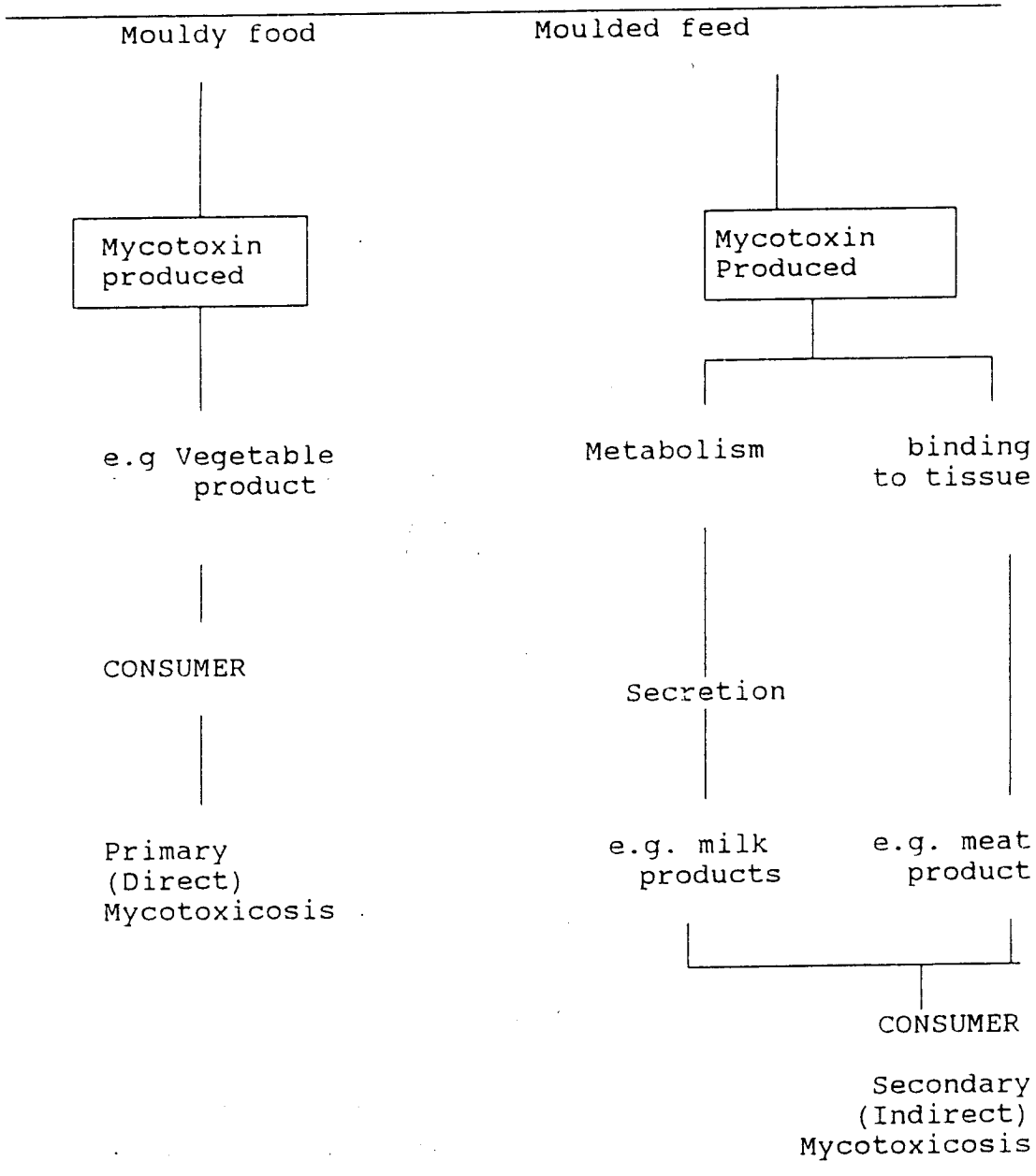
It is now widely appreciated that the presence of mycotoxin contamination in foods and feeds represents a considerable health problem to man, animals and poultry (Wogan, 1964; Coker, 1979). However, it is difficult to be precise about the group of illnesses (mycotoxicoses). When the contaminated plant food (e.g. maize and groundnuts) is eaten by humans and produces intoxication, this can be considered as primary mycotoxicosis. It is also possible for domestic animals and poultry to consume mycotoxins with their feed without showing overt signs of illness. The contaminated animal products (meat, milk, eggs) are then eaten by humans causing secondary mycotoxicoses (Strzelecki, 1980). In both the direct (primary) and indirect (secondary) sources, the toxic metabolites may enter the food chain via foods which the consumer may not even suspect could be contaminated (fig. 9).

Human exposure to mycotoxins is predominantly through the direct way but it can also occur through the edible products from animals that have consumed aflatoxin in the feed. When mammals ingest aflatoxin, the toxin can be passed through to the milk where it occurs as the metabolite aflatoxin M1. This discovery led to the Ministry of Agriculture in the United Kingdom, in 1981, deciding to ban the feeding of contaminated groundnut products to dairy cows because of the possible hazard to the health of milk-drinkers (Swindale, 1983).

The milk toxin (aflatoxin M1) is thought to be particularly important as young mammals including humans are more susceptible to aflatoxins. It has been suggested by Goldblatt and Stoloff (1983) and Hsieh (1983) that bovine milk animal product is the only tissue in which measurable levels of aflatoxins are likely to be found in market samples.

FIG. 9

PATHWAYS BY WHICH MYCOTOXINS MAY REACH THE CONSUMER THROUGH FOOD CHAINS.



Moss, 1977; Hsieh, 1983.

1.5.2. METABOLISM AND TOXICITY OF AFLATOXINS.

Xenobiotic metabolism is an immense area of study, and this is reflected in the range of chemical reactions the substrates can undergo during metabolism. The term "Xenobiotic" is derived from the Greek meaning "a substance foreign to living organism." Xenobiotic (drugs ingested either deliberately or unintentionally and mycotoxins) metabolism is normally divided into two phases : phase I or functionalisation reactions and phase II or conjugative reactions (Table 4). The phase I reactions create a chemically reactive functional group (such as - OH, $-NH_2$, -SH, -COOH etc.) on the molecule so that it is in the correct chemical state and can be attacked by the phase II (conjugative) enzymes. Thus, the phase II reactions are the "true detoxification" pathways (Gibson, 1980) and give products that account for the bulk of the inactive, water-soluble products of a xenobiotic excreted in bile or urine (Patterson, 1977).

Mycotoxin metabolism is discussed under three headings :
(1) detoxification, (2) toxic residues and the public health problem arising from the possible accumulation of these in the tissues of farm animals and poultry consuming toxin-contaminated feeds, and (3) metabolic activation and mode of action.

1.5.2.1. DETOXIFICATION.

This can be explained as the biotransformation of a toxin, usually by the microsomal mixed function oxidases (MFO) of the liver, to a metabolite that is non-toxic when ingested by a second animal of the same or different species or to a metabolite that results from the conjugation of the toxin with cysteine, glutathione, or glucuronic, taurocholic or sulphuric acid in a further microsomal reaction to form more polar compounds that are rapidly eliminated from the body into the urine or bile, for example (Patterson, 1977) (Table 4).

The aflatoxin molecule lends itself to biodegradation in at least six ways (fig. 10). Virtually all six of the possibilities have been described by various authors as being likely or actual modes of biodegradations (Wyllie and Morehouse, 1980).

Aflatoxin B_1 may be transformed in the liver into three hydroxylated metabolites that are at least theoretically capable of conjugation to polar compounds suitable for rapid elimination from the body. Aflatoxin B1 is metabolised in the liver cells and converted to classes of metabolites (aflatoxin M1, P1, Q1, B2a etc) (fig. 11).
These form free or conjugated primary metabolites, water-soluble conjugatives which can appear in urine and, metabolites that are covalently bound to cellular macromolecule adducts (Patterson, 1977).

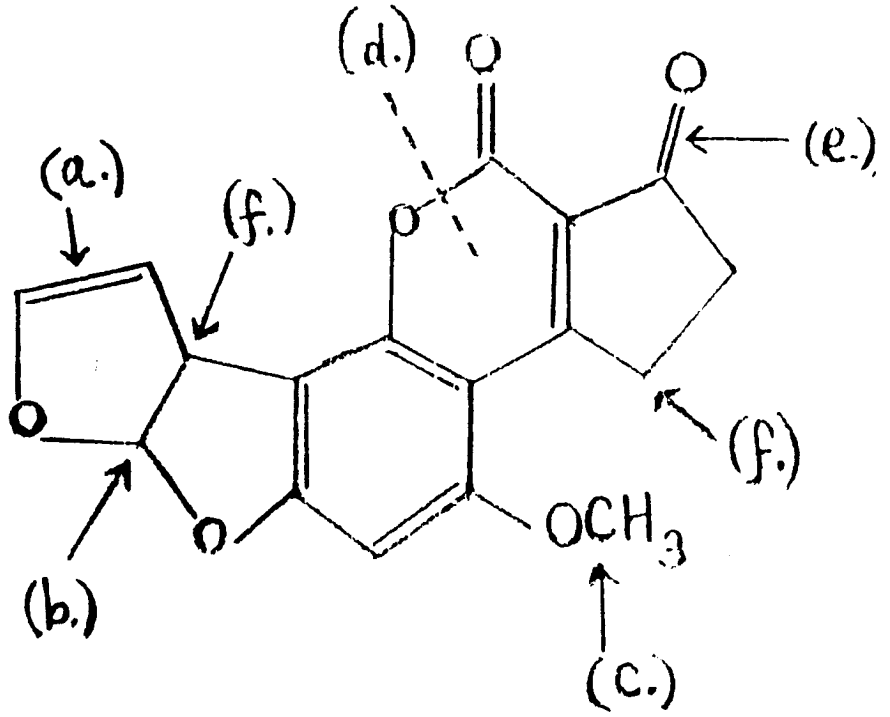
Table 4
PHASE I OR PHASE II METABOLISM REACTION.

PHASE I	PHASE II
Oxidation.	Glucuronidation/Glucosidation.
Reduction.	Sulfation.
Hydrolysis.	Methylation.
Hydration.	Acetylation.
Dethioacetylation.	Amino acid conjugation.
Isomerisation.	Glutathione conjugation.
	Fatty acid conjugation.
	Condensation.

Gibson, 1980.

Fig. 10

BIODEGRADATION OF THE AFLATOXIN B_1 MOLECULE

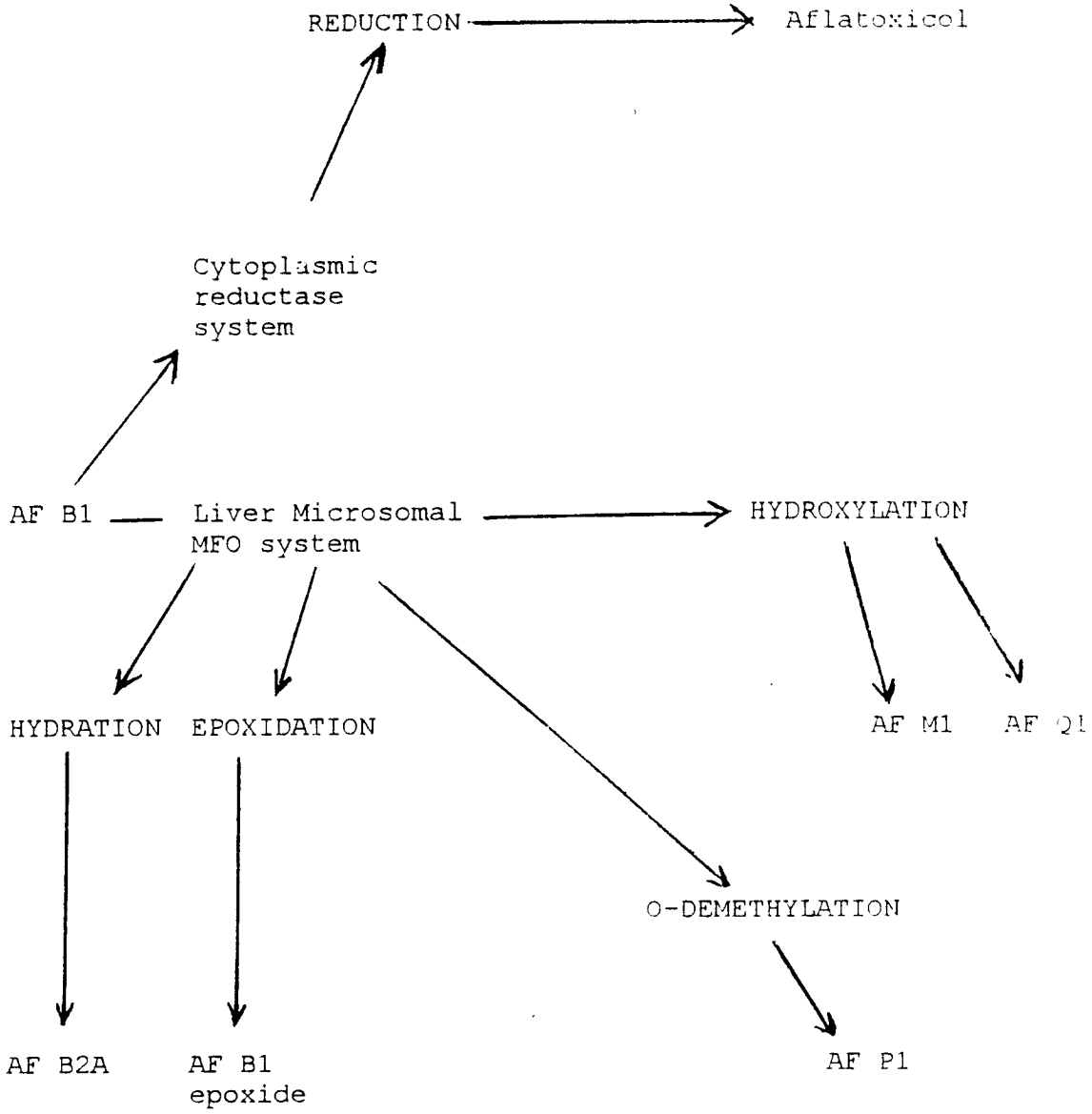


Note.

a. Hydrolysis and Epoxidation.
b. Bisfuranoid degradation.
c. Demethylation.
d. Hydrolytic fission
e. Reduction.
f. Hydroxylation.

Fig. 11a.

PATHWAYS BY WHICH AFLATOXIN B_1 MAY BE METABOLISED.



Moss, 1977; Hsieh, 1983.

FIG 11b.

BIOTRANSORMATION OF AF B1

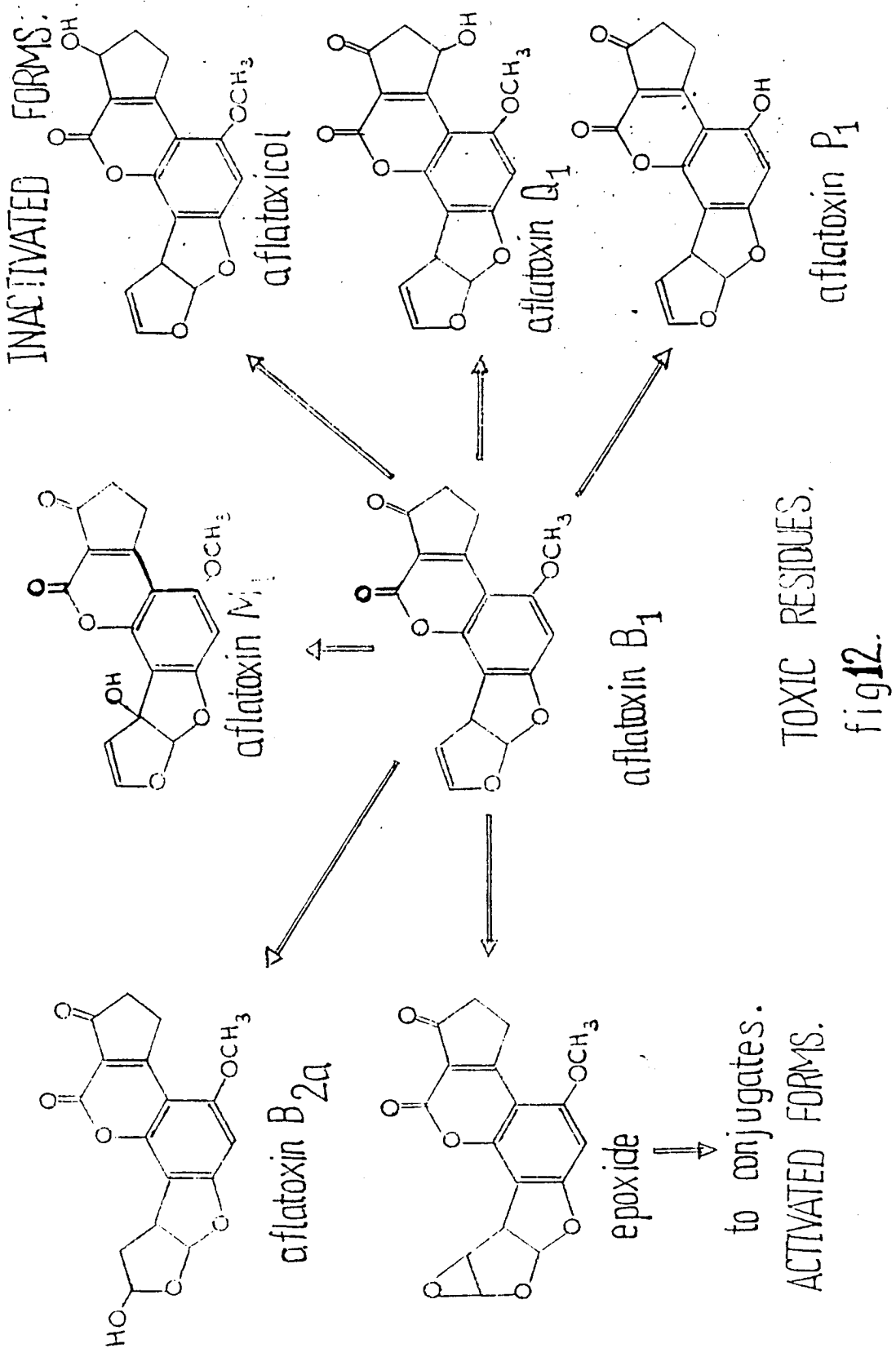


fig12.

To this extent, these reactions involve detoxification and the products are all appreciably less toxic than the parent toxin, except for aflatoxin M_1. Aflatoxicol is not, however, formed in the microsomal mixed function oxidase reaction but by an NADP-linked dehydrogenase of the cytosol (Campbell and Hayes, 1976). This reaction is reversible (fig. 11) and constitutes a "metabolic reservoir" of aflatoxin B1.

1.5.2.2 **TOXIC RESIDUES.**

Aflatoxin is metabolised more rapidly by some animal species than others (Patterson, 1977) and consequently in "slow-metabolising" animals, aflatoxin B1 can constitute a residue problem particularly in tissues of farm animals and poultry. The biotransformation of aflatoxin B1 to aflatoxin M1 by microsomal enzymes is to some extent a detoxification since conjugates of aflatoxin M1 have been identified in bile and urine, but because unconjugated aflatoxin M1 is almost as toxic as aflatoxin B1, it must still be regarded as a potentially toxic residue. A considerable public health problem exists in the secretion of aflatoxin M1 in the milk of dairy cows consuming rations contaminated with aflatoxin B1. It was Allcroft and Carnaghan (1963) who first demonstrated that if lactating cows were fed cattle cake rich in aflatoxin, their milk revealed only traces of aflatoxin B1 but demonstrated the presence of a related compound to which the name aflatoxin M1 was given.

1.5.2.3. **METABOLIC ACTIVATION.**

While a xenobiotic undergoing one of the reactions usually gives rise to a more polar metabolite, the metabolite is not always less toxic or pharmacologically inactive. This is especially true of metabolites resulting from oxidation, reduction and hydrolysis (Adamson, 1974). Metabolic activation differs from detoxification in that it involves biotransformation of the mycotoxin to at least one metabolite which is more toxin than the parent toxin and also plays a key role in the mechanism of toxin action (Patterson, 1977), e.g aflatoxin B1 epoxide. Examples of some xenobiotics whose metabolic products are more active than the parent compounds are shown in table 5.

The metabolism of aflatoxin B1 either to a more (metabolic bioactivation) or a less (detoxification) toxic product occurs through various biotransformation pathways (Patterson, 1977) and plays a prominent role in determining the toxicity of aflation B1 since it requires some metabolic activation to exert its activity (Campbell and Hayes, 1976).

The metabolic biotransformations for aflatoxin B1 are shown in Fig. 11a. The activity of aflatoxins B1, G1 and M1 derives from the unsaturation in the furan moiety as metabolic activation depends on the existance of the double bond. The epoxide is the most reactive and its reactivity with some biomolecules (RNA, DNA and glutathione) in the cells leads to toxicity on complexation and conjugation. The result is that, apart from the inhibition of nucleic acid, protein and lipid synthesis, glucose metabolism and clotting factor synthesis are also inhibited or depressed. Toxicity in the liver manifests itself through pathological changes and these include fatty infiltration, biliary proliferation, acute toxic necrosis and portal fibrosis (Hendrickse, 1985).

1.5.2.4. TOXICITY OF AFLATOXINS.

When referring to a toxic dose, it is necessary to specify the route of ingestion of the toxin. When considering toxic moulds in feeds, which is the subject of this study, it is the oral route which is relevant. The toxicity of a toxicant to a particular organism is usually expressed in terms of the LD_{50} (lethal dose). This value represents the amount of poison per unit weight which will kill 50% of the particular population of the animal species employed for the tests. The LD_{50} is commonly expressed as milligrams per kilogram (mg/kg). The quantitative expression of toxicity is only a convenient criterion for defining the acute toxicity of a toxicant as it does not take into account effects less severe than leading to death (Table 6).

Of all the mycotoxins known to affect poultry, aflatoxin is perhaps the most toxic of all. One of the underlying causes for the extreme toxicity of aflatoxin is the rapid absorption of the toxin from the gastrointestinal tract, which is evidenced by the appearance of aflatoxin in the blood immediately following ingestion of the toxin (Wyatt, 1991).

Most relevant studies previously undertaken ask the question, "At what exposure to a chemical toxicant will an organism die?" Although all species are constructed practically of the same molecules, they do not respond exactly alike because of age and sex, and other factors.It is these differences (individual or group) that result in the different toleration and toxic action and different lethal doses and lethal concentration (LC_{50}'s) when test populations are exposed to toxicants.

1.5.2.5. FACTORS AFFECTING THE TOXICITY OF AFLATOXINS IN POULTRY AND ANIMALS

It is generally accepted that the toxicity of aflatoxin is variable among species (mammals, poultry, and man) and within the same species. Factors, some particular to some strains, including environmental ones acting on the host, the genetic makeup and immune status of the host itself tend to influence the severity of the infection and the outcome of the disease.

1.5.2.5.a. **Dose of aflatoxin.**
The reaction of a host to aflatoxin toxicity depends on the amount (dose) of aflatoxin that is ingested. Patterson (1977) reviewed work on the relationship of dose of aflatoxin to the severity of pathological and histopathological changes induced. Although too high amounts are rare, several workers have noted withdrawal effects when excessive amounts are given. An increase in the amount of aflatoxin ingested by the host is usually accompanied by an increase in the severity of the disease. However, very heavy doses of aflatoxin produced lower mortality in chickens. It is possible that the excessive amounts of aflatoxin produce a host reaction resulting in feed refusal.

1.5.2.5.b. **Toxicity of aflatoxins.**
The toxicity of aflatoxins differs with aflatoxins B1 and M1 being the most toxic (Table 7.). With regard to aflatoxin produced in a laboratory it has been found that the most toxic of the parent aflatoxins, AF B1, is usually produced in smaller quantities than aflatoxins G1, G2 and B2 (Njapau, personal comm.).

1.5.2.5.c. **Site of development within the Host.**
Most aflatoxicoses develop within the liver of th p-1Xe host. Those that devel in other organs tend to cause less damage (Wyatt, 1991).

1.5.2.5.d. **Age and sex of Host.**
Younger animals are generally assumed to be more susceptible to aflatoxicosis than their older counterparts. Growth retardation was found to be more severe in 2-weeks-old than in 6-week-old chickens given similar doses of aflatoxin. However, in both animals and poultry, older animals raised aflatoxicosis-free are as susceptible or more susceptible than very young ones to similar doses of aflatoxin. Sex differences in xenobiotic action (Gibson, 1980) show that female rats required only half of the dose of barbiturate needed by male rats to induce sleep. This was due to the reduced capacity of the female to metabolise the barbiturates. Female rats are more resistant than male to both toxic and carcinogenic effects of aflatoxin and this tendency is observed even at low doses of aflatoxin. Sex was not considered since there appears to be no report that sex has an effect on resistance to aflatoxin.

Table 6.

ACUTE TOXICITY OF AFLATOXIN B_1.

SPECIES	LD_{50} (mg/kg)
Chicken embryo	0.025 mg/embryo
Duckling	0.35-0.46
1 day old rat	1.0
21 day old rat	5.5
Adult rat (male)	7.2
Adult rat (female)	17.9
Rabbit	0.3
30 day old hamster	10.2
Guinea pig	1.4
Sheep	1.0
Pig	0.6
Dog	0.5-1.0
Cat	0.6
Mouse	9.0
Chicken	6.3

Moreau, 1979

Table 7.

ACUTE ORAL TOXICITY OF AFLATOXIN IN DAY-OLD DUCKLINGS.

AFLATOXIN	LD_{50} (mg/kg BW)
M1	0.33
B1	0.36
G1	0.78
B2	1.70
G2	2.45

Wyllie and Morehouse, 1980.

1.5.2.5.e. **Breed of Host**

Most of the work on genetic resistance or susceptibility to aflatoxin toxicity has been done with commercial chicken breeds. This research project investigates the effects of aflatoxins on village chickens in Zambia. Genetic resistance to death due to aflatoxin need not necessarily be accompanied by a decrease in other pathogenic effects such as weight loss.

1.5.2.5.f. **Other factors**

It is often found that the environment and the nutritional status of the host affect the toxicity of aflatoxin.It has been shown,for example,that aflatoxins in the diet increase the severity of Eimeria tenella (Wyatt et.al.,1975;) and that malnutrition worsens aflatoxin toxicity of children (Bhat,1983;Samuel,1987).Higher crude protein in rations are protective against weight loss due to aflatoxin.

1.6. AFLATOXIN RESIDUES IN POULTRY AND HUMANS

For some time, there has been concern about the possibility of human exposure to aflatoxin B1 residues in food of animal origin (Rodricks and Stoloff, 1977). Several studies have been performed on aflatoxins and aflatoxin-residues in both poultry and farm animals. The residues could either be the aflatoxin consumed (aflatoxin B1 has been the one mostly extensively identified) or one of the metabolites (surveillance studies concentrate on aflatoxin M1 while experimentally aflatoxicol H1, aflatoxins P1, Qa and B2a have been identified as well) (Samuel,1987).

1.6.1. AFLATOXIN RESIDUES IN POULTRY AND ANIMALS.

The significance of animal-derived foods as sources of dietary aflatoxins for humans is a complex problem because of the possible retention of the parent toxins or their metabolites in animal tissue, milk or eggs from poultry. The retention of aflatoxin residues in animal and poultry tissue, following ingestion of contaminated diets, and their transmission into animal and poultry products have been a topic of study. Generally, these studies have indicated a correlation between dietary aflatoxin content and tissue residue concentration (Gregory III et.al., 1983).

A series of studies conducted in the U.S.A. (Jones,1975) on the effect of feeding graded levels of aflatoxin to swine, cattle and poultry on possible transmission of aflatoxin into milk, meat and eggs confirmed the results of earlier work, which were that the chemical and biological tests available at the time could not establish the presence of detectable aflatoxin levels in tissue and/or eggs. In a 1971 study, Keyl and Booth, using some recognised chemical methods, were unable to detect aflatoxin in meat from swine and cattle fed rations containing 0.8 and 1.0 µg/kg of aflatoxin, respectively. Further, eggs and meat from white Leghorn hens fed a ration of 2.7 mg/kg aflatoxin and the meat of broilers fed from one-day to 8 weeks of age on a ration containing 0.4 µg/kg aflatoxin contained no detectable aflatoxin. Aflatoxin has been detected in animal tissue only when the aflatoxin levels in the animal's diet have been relatively high (Allcroft and Roberts, 1968).