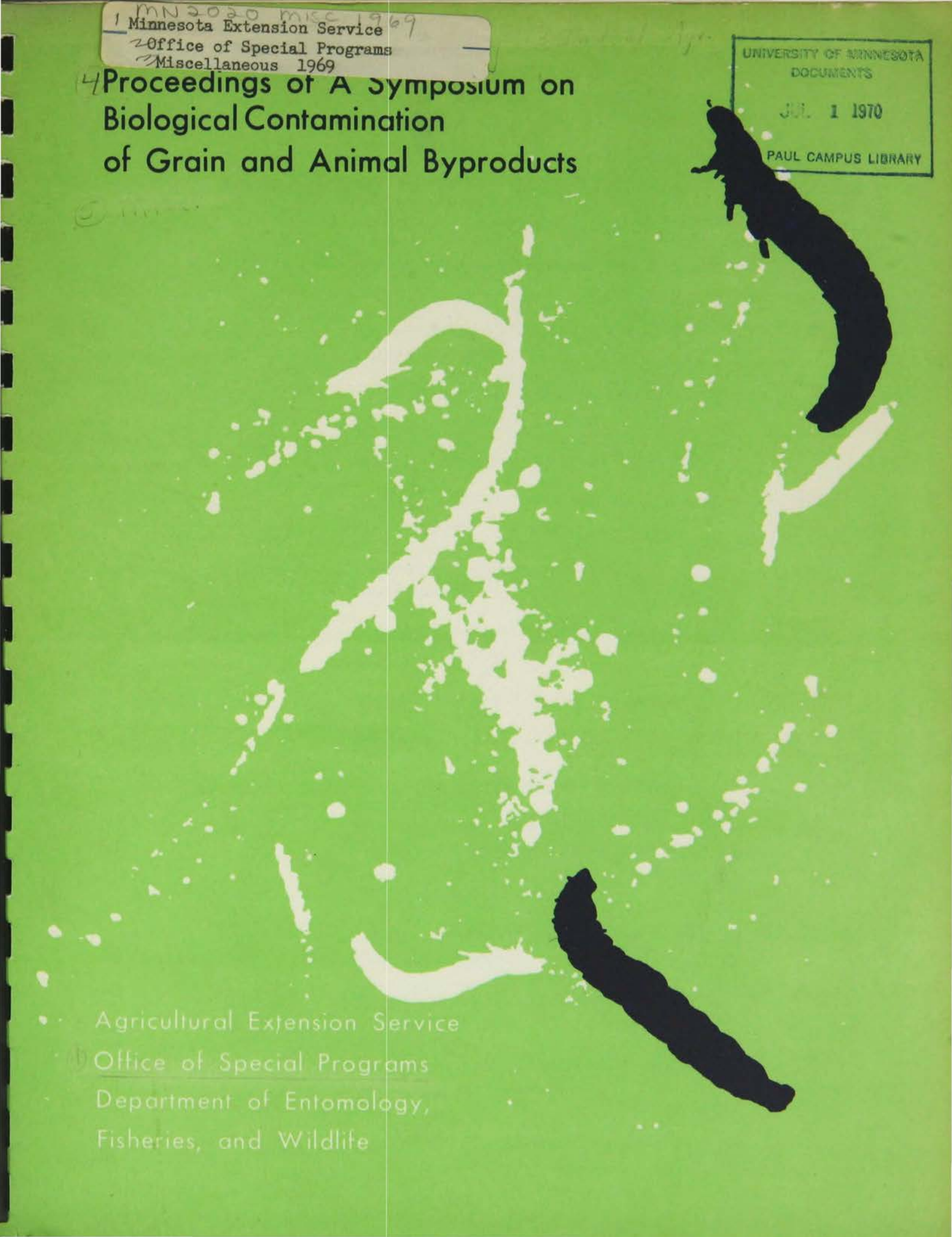
Proceedings of A Symposium on Biological Contamination of Grain and Animal Byproducts



Agricultural Extension Service
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Department of Entomology,
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BASIC MICROBIAL CONTAMINATION*

Edmund A. Zottola
Associate Professor and Extension Food Microbiologist
Department of Food Science and Industries

It is not very often a microbiologist has the opportunity to be the lead-off speaker at a symposium. Usually, we find ourselves at the end of the program or just before a lunch break and almost always running short of time. But today I am first and I hope we can set the proper mood for the program. The program committee felt that in order to develop an understanding of the topic under consideration, "Biological Contamination of Grain and Animal By-Products," that we first should explore the world of the microbe, that unknown area that we all know is there, and yet because we cannot see it we most of the time forget it or overlook it. Once an appreciation for the microbial world is developed, the importance of the other topics in this symposium will become more apparent. As we discuss our topic, the term food will include both human and animal food.

The microorganisms of major importance in the food industry include:

Bacteria - which are single celled living organisms - reproduction is primarily by binary fission size ranges 0.5 to 80 microns.

Yeasts - are single cell living organisms which usually reproduce by asexual budding and range in size from 5.0 to 10.0 microns.

Molds or fungi - are multicellular organisms with many morphological features, reproduction may be by sexual or asexual means.

Viruses or bacteriophage - are obligate parasites which grow only in living cells, intracellular parasites, extremely small 0.01 to 0.3 microns. When these parasites infect bacteria, they are called bacteriophage.

Microorganisms occur nearly everywhere in nature. They are carried by air currents from the earth's surface to the upper atmosphere. Even those indigenous to the ocean may be found many miles away on mountain tops. The soil is filled with them; there may be as many as 1 billion in a gram of soil from a fertile field. They are carried by streams and rivers into lakes and other large bodies of water; and if human wastes containing harmful bacteria are discharged into streams, diseases may be spread from one place to another. They occur most abundantly where they find food, moisture and a temperature suitable for their growth. The conditions that favor the survival and growth of many microorganisms are those under which man normally lives, so it is inevitable that we live among a multitude of microbes. They are in the air we breathe and the food we eat. They can be found

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on the surfaces of our body, in our intestinal tract, in our mouth, nose and other body openings. It is certainly fortunate that most microorganisms are harmless to us and that we have ways of resisting invasion by those that are potentially harmful.

One characteristic of microorganisms that is somewhat difficult to comprehend is their size. They are so small that it is not possible to use the normal dimensions of size, so microbiologists use a unit called a micron to measure bacterial size. A micron is equivalent to 1/25,000 of an inch and as was mentioned earlier the size varies from 0.5 to 80 microns. Indeed they are tiny - if we had a cubic inch solid with bacteria that cubic inch would contain over 8 trillion cells! In order to "look at" bacterial cells a microscope that magnifies approximately 1000 times is used. How much magnification is that? To illustrate how much, if we were to magnify the height of a man 6 feet tall 1000 times, he would be over 1 mile high!

Today, rather than discuss each type of microorganism important to the food and feed industry we will confine our comments mainly to bacteria as many of the concepts we are trying to illustrate with bacteria apply equally as well to the other organisms we named earlier.

Bacteria come in three basic styles, round, rod-shaped, and spiral. The round cell or cocci may occur singly, in pairs, in chains or in clusters. The rod-shaped organism may also be found singly, in pairs and sometimes in chains. In the food industry we are not too concerned at the moment with spiral-shaped organisms. How do these different shaped cells look under a microscope -- slides will give you an idea as to streptococcus, staphylococcus, small rods, and spore-forming rods.

Some of the rod-shaped bacteria form what is known as an endospore or spore. One cell gives rise to one spore and this in turn under proper conditions will return to one cell. The spore is considered to be a resting or dormant stage of the cell - and in this state the organism is much more resistant to adverse effects - heat, chemicals, desiccation, drying, and so on. The spore is a means of assuring the species survives. Spore-forming bacteria have been recovered from food canned over 100 years ago!

Another confusing aspect of microbiology and one we should spend a minute or two on is how bacteria are named. A binomial nomenclature is used, that is two words. The first word identifies the genus and usually reflects either where it was first found or by whom it was first found. The second word identifies the species and this also may indicate either the discoverer or the location. For example:

Salmonella pullorum

Streptococcus lactis

Lactobacillus bulgaricus

Salmonella st. paul

There are more than 1200 different species of salmonellae and each has its own identifying name. Let us turn now from how bacteria are named to how they grow.

When we talk about bacterial growth, we really mean reproduction, that is, growth is not an increase in the size of the individual bacterium but rather an increase in the number of individual bacteria. Therefore, when talking about microorganisms, growth and reproduction usually mean the same thing and are used interchangeably. Reproduction or growth occurs by binary fission, one becomes two, two-four, four-eight and so on, growth occurs in a geometric progression. Another concept of bacterial growth to keep in mind is their ability to utilize foodstuff and grow under ideal conditions. Under such a situation one cell can consume food equivalent to its body weight in one minute and can reproduce in 10 minutes. By comparison, a pig is a slow eater, it takes about 20 days to consume food equivalent to its body weight and somewhat longer to reproduce. If a cow had the ability to utilize foodstuff in the same manner as a microorganism, she could consume several tons of feed a day. I am sure that would make a lot of feed dealers happy!

Before we continue our discussion on growth we should become familiar with the term microenvironment. The microenvironment is the area immediately adjacent to the organism which influences its activity. Since microorganisms are extremely small, the microenvironment is also. The conditions in the mass of material that is the microenvironment may appear to be undesirable for bacterial growth, yet there may be localized conditions throughout this mass which provide an ideal microenvironment for bacterial growth. Keep this concept in mind as we continue to talk about bacterial growth.

What are the factors that influence the growth of bacteria? These are:

1. Air or oxygen
2. Microbial inhibitors
3. Acidity or alkalinity (pH)
4. Moisture - available water
5. Nutrients - available food
6. Temperature

We will develop each of these factors further as we continue.

How does the presence of air affect growth:

Aerobic organisms need air or oxygen to grow, examples, Bacillus species, Pseudomanads, molds.

Anaerobic microorganisms grow only in the absence of free oxygen, example Clostridium species.

Facultative organisms have the ability to develop with or without air or oxygen. Examples, coliform organisms, staphylococci, salmonellae and many others. In fact, so many bacteria are facultative, that oxygen control is not an effective means of controlling bacterial growth.

Microaerophilic organisms grow best under conditions of reduced oxygen tension.

Bacterial activity is markedly affected by the presence or absence of microbial inhibitors.

Germicides and Sanitizers - these are chemicals compounded to inhibit or destroy microorganisms. Their presence in an environment certainly does influence growth.

Antibiotics - these are compounds produced by other microorganisms which prevent growth.

Presence of other microorganisms - some bacteria grow more rapidly than others and thus are able to inhibit the activity of the others present.

By-Products of microbial growth - antibiotics are by-products of microbial growth, some lactic acid producing organisms will develop sufficient acid that they inhibit their own growth.

Salt, fatty acids and other inhibitors - these are compounds usually added to material to prevent growth of microorganisms such as sodium benzoate to prevent mold growth.

Another factor influencing growth is the pH or the acidity or alkalinity of the system. Some microorganisms are highly sensitive to changes in pH. Others will grow over a wide range. The optimum pH is 6.8 - 7.0. Few microorganisms grow below pH 4.5 or above pH 10.0. Yeasts and molds grow over a wide pH range. This fact is utilized when enumerating these microorganisms. A low pH (below 4.0) is used to preserve many foods.

Metabolic activity can only occur in the presence of moisture. This is true of any living organism whether it be man, animal, plant or microorganism. Microorganisms require moisture to serve as solute for dissolving of food material; to remove waste products from the system; and water enters into many of the chemical reactions necessary for life processes. One method of preventing bacterial activity is to remove moisture from the system by drying. While this prevents bacterial growth, it should be emphasized that drying does not destroy most bacteria, indeed it preserves them and once water is returned to the system the activity can and does resume.

We have talked about the influence of oxygen, inhibitors, pH, available water - now what about the availability of food, how does this affect microbial activity? The food which microorganism depends upon for growth must contain:

1. An energy source
2. Sources of substances which make up cell protoplasm
3. Essential substances, such as minerals, vitamins and so on.

   These are necessary to maintain chemical balance within the cell and essential for normal functioning of some of the reactions which occur in the cell system.

Microorganisms vary in their nutritional requirements all the way from the simplest of materials to extremely complex compounds. Some molds and other microorganisms such as some soil and water bacteria require only ammonium salts, glucose and trace amounts of several inorganic ions in water solution for growth. Other microorganisms require a very complex food for growth which should include vitamins, minerals, amino acids and other growth factors. And as we indicated earlier water is necessary for growth. It appears then that there is very little material on this earth that will not support the growth of some type of microorganism.

The final factor we are going to discuss today and the one most often used to control bacterial activity is temperature. Temperature variation can be used to enhance or inhibit activity or completely destroy bacteria. How does this affect growth? In general, warmer temperatures speedup growth, cooler temperatures slow it down. This organism has the ability to grow over a wide temperature range. Notice how the generation time increases as the temperature decreases indicating bacterial activity is slowed down greatly by a reduction in temperature. This information also demonstrates how refrigeration helps to preserve food for a time.

It is possible to enhance bacterial growth by allowing the organism to remain at favorable temperatures. This is done every time we incubate a culture, carry out a fermentation or allow food to spoil when it is left in a warm room. Growth can be inhibited by reducing temperature, this was just illustrated. Microorganisms can be destroyed by temperature increases. Before we discuss this let's define some terms used to describe bacterial destruction.

Sterilization - refers to complete destruction of all living organisms in a system.

Sanitization or sanitation - indicate destruction or removal of unwanted or undesirable microorganisms in a system. These may be either pathogenic or spoilage organisms.

Disinfection - is a term that describes the destruction of pathogenic microorganisms.

These terms do not mean the same thing and should not be used interchangeably.

Microorganisms vary in their ability to survive heat treatments. A treatment has to be designed to destroy those organisms with which we are concerned. If sterilization is desired, then the treatment should be such that all organisms including spore formers are destroyed. If the material treated is heat liable, then lower temperatures should be utilized to destroy pathogenic organisms. Thus pasteurization of milk is based on the destruction of Coxiella burneti, the most heat resistant pathogenic organism associated with milk. Egg pasteurization is designed to kill any salmonellae that may be in the liquid egg. Other examples could be cited, each is designed to produce a desirable end product that is free from pathogenic and spoilage microorganisms.

We have briefly reviewed some of the factors affecting bacterial growth - now what types of bacteria are we concerned about? I like to divide bacteria roughly into 3 classes or types:

1. Useful bacteria
2. Spoilage bacteria
3. Pathogenic bacteria

USEFUL BACTERIA

Although many people believe otherwise, most forms of bacteria do not produce disease. They live in various places in nature, growing wherever they find the proper conditions. In some cases, their activities may not significantly affect the surrounding environment; in others, they perform changes of immeasurable benefit.

Many thousands of useful activities are performed by bacteria including: production of acid and flavor in the manufacture of many types of food; fixation of atmospheric nitrogen in soil; production of vinegar from alcohol; decomposition of waste; and buildup of soil through various activities.

SPOILAGE BACTERIA

In their attempt to live and perpetuate themselves, many bacteria produce changes in food products that damage flavor and composition. They can sour milk, spoil meat, turn vinegar bitter, and ruin many other food items. Therefore, from the time a food is produced and processed, there is a constant race between the producer, the processor, the consumer, and the bacteria. If the bacteria win, the food is damaged or spoiled.

PATHOGENIC BACTERIA

A relatively small proportion of bacteria can produce diseases and are a constant hazard to man, animals, and plants (the hosts). They produce such diseases by growing on or in certain tissues of the host and, thereby, injuring him. Or they produce harmful poisons called toxins on or in the host or in foods that the host eats later.

In conclusion what can we do to keep unwanted organisms out of our products? Let's return to factors necessary for bacterial growth.

1. Temperature - control
2. Moisture - remove
3. Food - remove
4. Inhibitors - use on equipment
5. pH - adjust
6. Oxygen or air - not applicable

Control these factors and we can prevent one microbe from becoming millions.

We become aware of bacteria not by their appearance but by the changes in our environment that they cause. These changes, either desirable or undesirable, are due to bacterial growth and can be controlled.

INFESTATION OF STORED GRAIN BY INSECTS AND MITES[1]

R. N. Sinha
Research Station, Canada Department of Agriculture, and
Faculty of Graduate Studies, University of Manitoba
Winnipeg 19, Manitoba

Introduction

Each year insects and mites reduce profits on farms and in the food industry through contamination. Infestation is frequently not apparent until outbreak levels have been reached, at which time damage is often irreparable and losses are heavy. Losses due to the depredation of storage pests include the absolute loss arising from destruction of the food, loss associated with aesthetic standards, and loss due to the cost of applying preventive or control measures. For example, infested grain is hard to sell and may be downgraded. Moreover grain buyers are reluctant to buy or may even be prevented from accepting infested grain because of the risk of infesting storage and transportation facilities. There are also losses in time, convenience, and patronage that are too intangible to assess.

In most temperate countries losses associated with aesthetic, sanitary and legal standards are usually of greater importance than the actual destruction of food by insects, mites, and rodents that is so prevalent in the warmer countries of the world. However, even in these developing countries, there are signs that as pest control becomes more widely practiced and food becomes more plentiful, increased emphasis will be devoted to higher sanitary and aesthetic standards.

Feeding habits, distribution and abundance

Over 100 species of insects and mites attack stored grain and foodstuffs in North America. Some invade the whole kernels whereas others only attack broken kernels. There are also species that feed almost exclusively on either germ, endosperm, grain dust, or dockage. Most species also feed on those field fungi associated with freshly harvested grain and on certain species of storage fungi in aging grain. These insects and mites can be divided into the following four major groups according to feeding habits:

Whole kernel feeders -- rice weevil, Sitophilus oryzae (L.); granary weevil, S. granarius (L.); lesser grain borer, Rhyzopertha dominica (F.); angoumois grain moth, Sitatroga cerealella (Oliv.). These insects, which cause the greatest economic losses, can penetrate most types of cereals. However, we have recently observed that the granary weevil and the lesser grain borer cannot penetrate the hulls of major oat varieties grown in Canada and the Northern U.S. (Sinha unpublished):

Germ feeders -- rusty grain beetle, Cryptolestes ferrugineus (Steph.); flat grain beetle,

[1]Contribution No. 363, from Canada Department of Agriculture, Research Station, Winnipeg 19, Manitoba.

C. pusillus (Schön.); Indian-meal moth, Plodia interpunctella (Hf.); grain mite, Acarus siro (L.).

Fungivores--foreign grain beetle, Ahasverus advena Waltl.; hairy fungus beetle, Typhaea stercorea (L.); mushroom mite, Tyrophagus putrescentiae (Schr.).

Broken kernel and grain-dust feeders: saw-toothed grain beetle, Oryzaephilus surinamensis (L.); confused flour beetle, Tribolium confusum Duv.; red flour beetles, T. castaneum (Hbst.). A species often belongs to more than one group. For example, some bran beetles are both broken grain feeders and fungivores.

If the insects are grouped according to cereals or food products, (Fig. 1) stored corn in the corn belt is infested by 6 species of insects, including the saw toothed beetle, the flat grain beetle and the red flour beetle; stored rice in the South is attacked primarily by the dreaded rice weevil. This weevil infests maturing grain in the field (Cotton and Ashby 1952). The confused flour beetle, the red flour beetle, the grain mite, and the mushroom mite infest milled cereal products in flour mills. Usually several species of arthropods occur in a stored grain infestation.

In any agricultural region usually not more than 12 species account for over 95% of the arthropod population in stored grain. The commonest pests in each crop within geographical areas are illustrated in Figure 1. The groupings are tentative because infestation records are incomplete for some areas.

The saw-toothed grain beetle, rusty grain beetle, and the flat grain beetles are the commonest insect pests of stored grain in North America. They do less direct damage to grain kernels than the whole grain feeders, but through infestation these three species most frequently cause grain to heat and spoil whether it is stored in Manitoba, Washington, Minnesota or Kansas.

Physical limits and rates of increase of insects

It is well known that appropriate temperatures and moisture contents are necessary before serious arthropod infestation can occur. The ranges of physical limits and the intrinsic rates of increase of the most important stored grain insects (Howe 1965) are summarized in Figure 2. Since the physical limits were established under controlled conditions in the laboratory they may not be directly applicable to the field. However, they are useful for purposes of comparison and may help to interpret field data.

Ecology of infested grain bulks

Figure 3 illustrates interrelations among insects, mites and microorganisms on the one hand and deterioration and bio-contamination of stored grain on the other. Ecological studies with stored grain on farms carried out by Cotton, Walkden, and Wilber in Kansas, (Walkden 1951, Cotton et al. 1960) and Sinha and Wallace (Sinha and Wallace 1966b) in Manitoba have revealed striking similarities in the development of infestations in grain bulks. In the fall, relatively dry, newly threshed grain (about 14% moisture content) with a natural in-

festation of the field fungus Alternaria stored in a farm granary often becomes infested with insects and mites. Infestation originates from external sources and probably begins in small pockets usually deep inside the bulk; the commonest insect species at this stage are the saw-toothed grain beetle and the rusty grain beetle as illustrated in Figure 4. By winter, due to heavy breeding of insects the grain temperature increases, moisture becomes redistributed along the surface of the grain bulk, and the grain is invaded by (1) the storage microorganisms, Penicillium, Aspergillus, Absidia, Streptomyces, and bacteria; (2) fungivorous and parasitic insects and mites, e.g. the foreign grain beetle and other fungus beetles, and mesostigmatic and acarid mites. By late winter or early spring the common seed-borne field fungus, Alternaria, has usually been replaced by storage fungi and many seeds lose their germinability.

Watters (unpublished) has shown in a detailed laboratory study of the locomotary activity of the rusty grain beetle that the insect generally migrates vertically from the dry to the damp grain and emigrates from the grain heavily infested with storage fungi. Insect and mite infestations are usually confined to the top foot of the bulk whereas fungi, especially the Aspergillus and Penicillium groups, invade the entire column. The grain column below the top of the hot spot dries during winter. The downward migration of arthropods from the top damp layer to the deeper areas becomes limited by low moisture and often high temperature whereas the horizontal migration is restricted by the low temperature of the surrounding grain mass. Occasionally population explosions occur manifested by a sudden many-fold increase of insects and mites after which the hot spots dry and cool. In spring, insects spread from overcrowded quarters to develop pockets of infestations in warm, moist layers at the top of the grain mass.

Experimental studies on arthropod-fungus interrelations

During studies in both the field and the laboratory we have observed numerous examples of close association between insects, mites, and fungi in stored grain. (Sinha and Wallace 1966). Other workers (Griffiths et al. 1959, Sikorowski 1964) have also observed that some species of insects and mites feed and reproduce on certain fungi, whereas other species of arthropods starve and die when exposed to the same fungi. We have devoted considerable effort during the past 10 years in studying the nature of interactions among certain seed-borne fungi and the major stored-product insects and mites as this information was deemed necessary to determine the causes of deterioration of stored grain. A few highlights of our research follow.

Newly hatched larvae of the rusty grain beetle were fed on individual species of field and storage fungi grown on potato-sucrose agar, at 33° + 1°C and 75 + 2% RH, for about 3 days. Striking differences in larval size were observed. Figure 5 shows that very large larvae, compared to those grown on wheat kernels (J) were obtained when the insect was grown on Nigrospora sphaerica (A), Alternaria tenuis (B) or Helminthosporium sativum (C) and larvae of above-average size were produced on either Mucor sphaerosporus (F) or Aspergillus flavus (H). Larvae fed on either Penicillium terrestre (N) or Aspergillus fumigatus (O) were small.

Over 30 common species of stored product insects and mites have now been tested for ability to reproduce on different species of common seed-borne field and storage fungi. The results of these experiments (e.g. Sinha 1964b, 1968) and those of other workers (Griffiths et al. 1959, Sikorowski 1964) have allowed us to divide the common North American seed-borne fungi into 4 classes according to their suitability as food for grain insects and mites (Table 1). The fungi most suitable for the insects are all field fungi. Because of their predominance during the early stages of grain storage they likely play an important role (along with moisture and temperature) in causing insect outbreaks.

Mites on stored grain

Although both insects and mites occur in stored grain and foodstuffs, the latter are often overlooked as agents of bio-contamination. This is because mites are scarcely visible to the naked eye and their direct damage is less dramatic than that of insects. Nevertheless, in the cool and humid areas of North America mites can be a serious problem to the grower, processor, and consumer of grain. Recent information on their interactions with many common seed-borne fungi suggests that they play an important role in contamination of grain bulks.

When present in large numbers mites give a characteristic pungent, "musty" smell, to foodstuffs. Because they are closely associated with seed-borne fungi, they often disseminate microbial spores (Sinha and Wallace 1966a, 1966b; Griffiths et al. 1959). Information on the distribution and abundance of stored-grain mites is incomplete in the United States since no comprehensive survey or long-range ecological study has been conducted. In Canada, however, (Sinha 1964a, Sinha and Wallace 1966a) the pathways of infestation by mites in grain bulks and the complex interrelations among grain, fungi, and mites have been investigated.

The most important mite species in North America are the grain mite, Acarus siro L., which feeds on the germ of the grain kernel and cheese, the mushroom mite, Tyrophagus putrescentiae (Schr.), which feeds on fungi, cheese and other foods, and the long-haired mite, Glycyphagus destructor (Schr.) which feeds on grain dust and fungi.
They are found most frequently infesting a wide variety of stored products, especially grain and grain products, dried fruit, cheese and mushrooms (Krantz 1955, Hilsenhoff and Dicke 1963, Sinha 1964a). A fully grown mite, which is pale whitish in color, is usually from 0.5 to 1 mm long; the egg, larval, and nymphal stages are usually not visible without magnification. Under favorable conditions, i.e. moisture content of the grain above 15% and temperatures near 10°C (50°F) they multiply rapidly. Two predators of acarid mites, the cannibal mite, Cheyletus eruditus (Schr.) and the brown grain mite, Androlaelaps casalis (Berl.) are also common in grain in Canada and the United States. In a laboratory study of the relationship between the grain mite and its predator, cannibal mite, Solomon (1962) found that at low temperatures, 3° to 10°C, the grain mite population increased while its predator, did not. Conditions that are too dry or too warm for the grain mite allow the cannibal mite to survive and increase but food must be available. He concluded that only under the most ideal conditions could the cannibal mite eliminate the grain mite in a grain storage.

Prevention and control of infestations

Insect outbreaks are caused by a combination of the following factors: warm weather during harvest which results in high initial storage temperature of grain, successive occurrence of mild winters, prolonged stockpiling of grain in unclean granaries, the heat generating ability of insects and fungi, uneven distribution of temperature and moisture in grain bulks. Mite outbreaks occur at low low temperatures if the grain is harvested with high moisture content and harbors abundant field fungi. Although it is not feasible to change the climate, much can be done to safeguard stored grain and foodstuffs. First, to preserve grain from insect and mite damage high standards of sanitation and good storage management should be practiced. Second, grain can be made less attractive to these pests by efficient drying, cleaning and application of grain protectants and fumigants. Third, research efforts should also be directed towards the development of insect resistant cereal varieties.

REFERENCES

Cotton, R. T. and W. Ashby. 1952. Insect pests of stored grains and seed. pp. 629-639. In Insects of the year, Book of Agriculture, 1952, USDA, Washington, D. C.

Cotton, R. T., H. H. Walkden, G. D. White, and D. A. Wilbur. 1960. Causes of outbreaks of stored-grain insects. Agr. Expt. Sta., Kansas State Univ. & USDA, Manhattan, Bull. No. 416, 35pp.

Griffiths, D.A., A. C. Hodson, and C. M. Christensen. 1959. Grain storage fungi associated with mites. J. Econ. Entomol. 52: 514-518.

Hilsenhoff, W. L. and R. J. Dicke. 1963. Effects of temperature and humidity on cheese mites with review of literature. USDA Marketing Res. Rept. No. 599, 46pp.

Howe, R. W. 1965. A summary of estimates of optimal and minimal conditions for population increase of some stored-products insects. J. Stored Prod. Res. 1: 177-184.

Krantz, G. W. 1955. Some mites injurious to farm stored grain. J. Econ. Entomol. 48: 754-755.

Sikorowski, P. P. 1964. Interrelation of fungi and insects to deterioration of stored grains. Inst. Agr. Sc., Washington State Univ., Tech. Bull. No. 42, 35pp.

Sinha, R. N. 1964a. Mites of stored grain--ecology and survey. Proc. Entomol. Soc. Manitoba 20: 19-33.

Sinha, R. N. 1964b. Ecological relationships of stored products mites and seed-borne fungi. Proc. First Internat. Congr. Acarology, Acarologia 6: 372-389.

Sinha, R. N. 1968. Adaptive significance of mycophagy in stored-product Arthropoda. Evolution 22: 785-798.

Sinha, R. N. and H. A. H. Wallace. 1966a. Association of granary mites and seed-borne fungi in stored grain and in outdoor and indoor habitats. Ann. Entomol. Soc. Amer. 59: 1170-1181.

Sinha, R. N. and H. A. H. Wallace. 1966b. Ecology of insect-induced hot spots in stored grain in Western Canada. Res. Popul. Ecol. 8: 107-132.

Solomon, M. E. 1962. Ecology of the flour mite, Acarus siro L. (=Tyroglyphus farinae DeG.) Ann. appl. Biol. 50: 178-184.

Walkden, H. H. 1951. Farm storage of cereal grains. Milling Productions, October, 1951.

Table 1. Common seed-borne fungi grouped according to suitability as food for stored-product insects and mites.

I.	Generally favorable, rarely poor
	Nigrospora sphaerica (Sacc.) Mason
	Alternaria tenuis sensu Wiltshire
	Cladosporium cladosporicides (Fres.) Sacc.
	Mucor sphaerosporus Hagem
	Curvularia tetramera (McKenney) Boedijin
II.	Moderately favorable, occasionally poor
	Stemphylium Botryosum Wallr.
	Mucor silvaticus Hagem
	Trichothecium roseum Lk.
	Aspergillus flavus Lk.
	A. glaucus Group[b]
	A. repens DeBary
	Fusarium moniliforme Sheld.
	Penicillium cyclopium Westl.
	P. chrysogenum Thom[a]
	P. herquei Bain & Sartory
	P. spinulosum Thom[a]
	P. canescens Sopp[a]
	P. digitatum Sacc.[a]
III.	Generally poor, occasionally favorable
	Absidia orchidis (Vuill.) Hagem
	Rhizopus nigricans Fischer
	Trichoderma viride Pers. ex Fr.
	Helminthosporium sativum P., K., & B.
	Penicillium funiculosum Thom
	P. terrestre Jens.
	Aspergillus versicolor (Vuill.) Tiraboschi
	Chaetomium funicola Cooke
	Scopulariopsis brevicaulis (Sacc.) Bain.

IV Unfavorable, rarely acceptable

Streptomyces griseus (Krainsky) Waksman & Henrici

Aspergillus fumigatus Fres.

A. niger Van Tiegh.

A. ochraceus Wilh.

A. parasiticus Speare[b]

P. implicatum Biourge[a]

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[a] not tested on insects.
[b] not tested on mites.

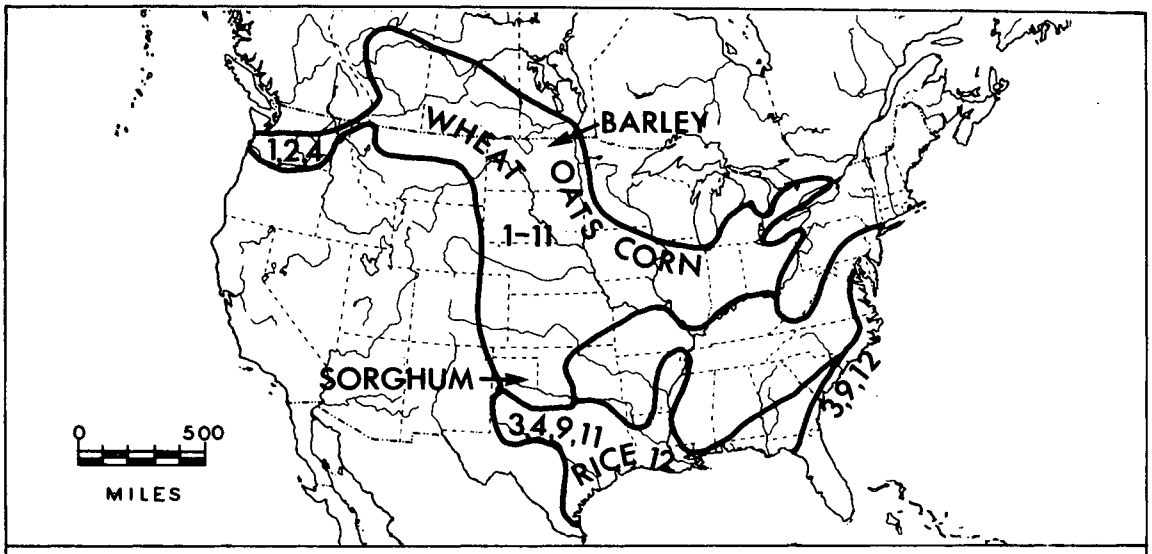
Fig. 1. Major insect pests of stored grain in the cereal-producing regions of Canada and the United States.

Fig. 2. Physical limits and rates of increase of the major stored grain insects in Canada and the United States.

Fig. 3. Pathways of infestation and contamination of grain by insects, mites and micro-organisms.

Fig. 4. A schemetic diagram of the initiation and development of an insect-induced hot spot in a farm-stored grain bulk. The diameter of the vertical columns represents the relative sizes of insect populations at various depths. The relative abundance of the main types of insects, mites and fungi in samples are shown in the two-toned circles.

Fig. 5. Relative sizes of 3-day old larvae of the rusty grain beetle, *C. ferrugineus* fed on: (A) *Nigrospora sphaerica*, (B) *Alternaria tenuis*, (C) *Helminthosporium sativum*, (D) *Fusarium moniliforme*, (E) *Stemphylium botryosum*, (F) *Mucor sphaerosporus*, (G) *Chaetomium funicola*, (H) *Aspergillus flavus*, (I) *A. ochraceus*, (J) Wheat kernel with exposed germ, (K) *Cladosporium cladosporioides*, (L) *Rhizopus arrhizus*, (M) *Aspergillus versicolor*, (N) *Penicillium terrestre*, (O) *Aspergillus fumigatus*, (P) Potato-sucrose agar.



1 SAW-TOOTHED GRAIN BEETLE	7 HAIRY FUNGUS BEETLE
2 RUSTY GRAIN BEETLE	8 INDIAN MEAL MOTH
3 FLAT GRAIN BEETLE	9 RICE WEEVIL
4 RED FLOUR BEETLE	10 GRANARY WEEVIL
5 CADELLE	11 LESSER GRAIN BORER
6 FOREIGN GRAIN BEETLE	12 ANGOUMOIS GRAIN MOTH

Fig. 1

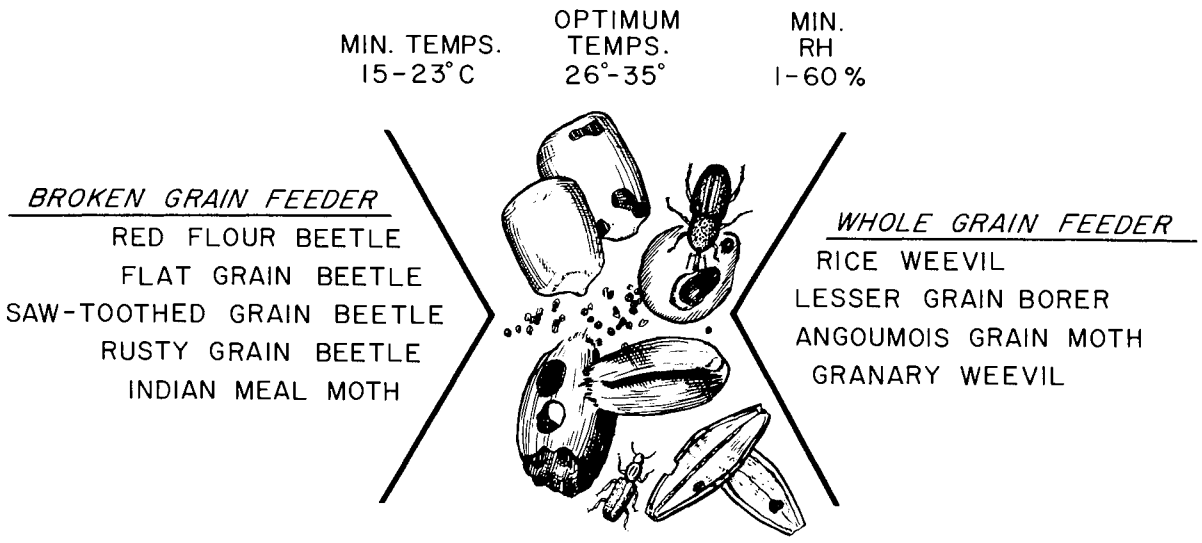


Fig. 2

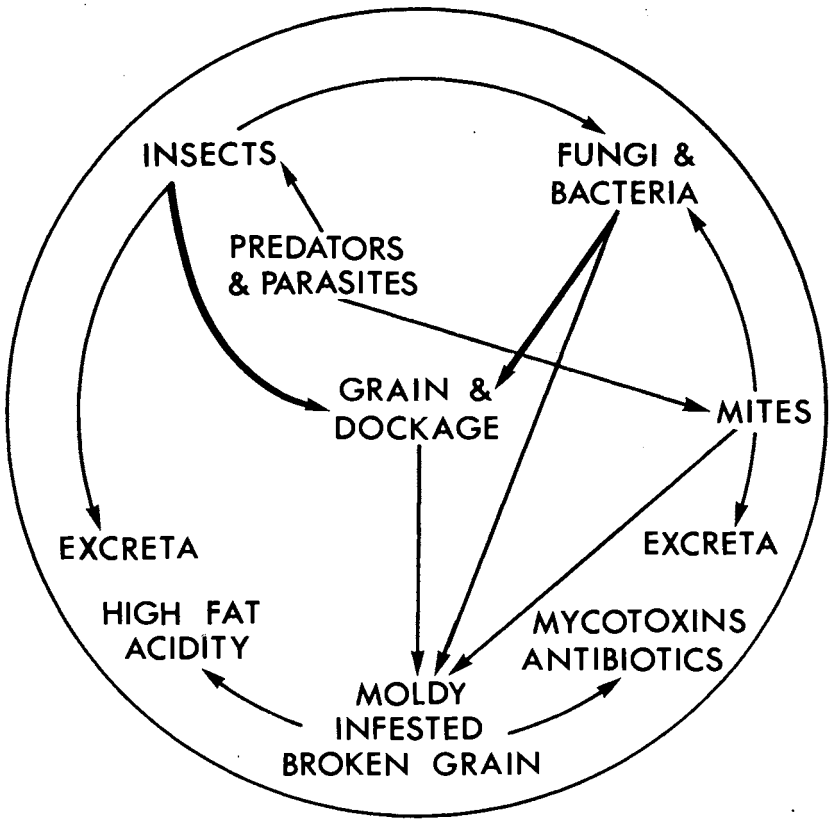

INSECTS
FUNGI &
BACTERIA
PREDATORS
& PARASITES
GRAIN &
DOCKAGE
MITES
EXCRETA
EXCRETA
HIGH FAT
ACIDITY
MYCOTOXINS
ANTIBIOTICS
MOLDY
INFESTED
BROKEN GRAIN

Fig. 3

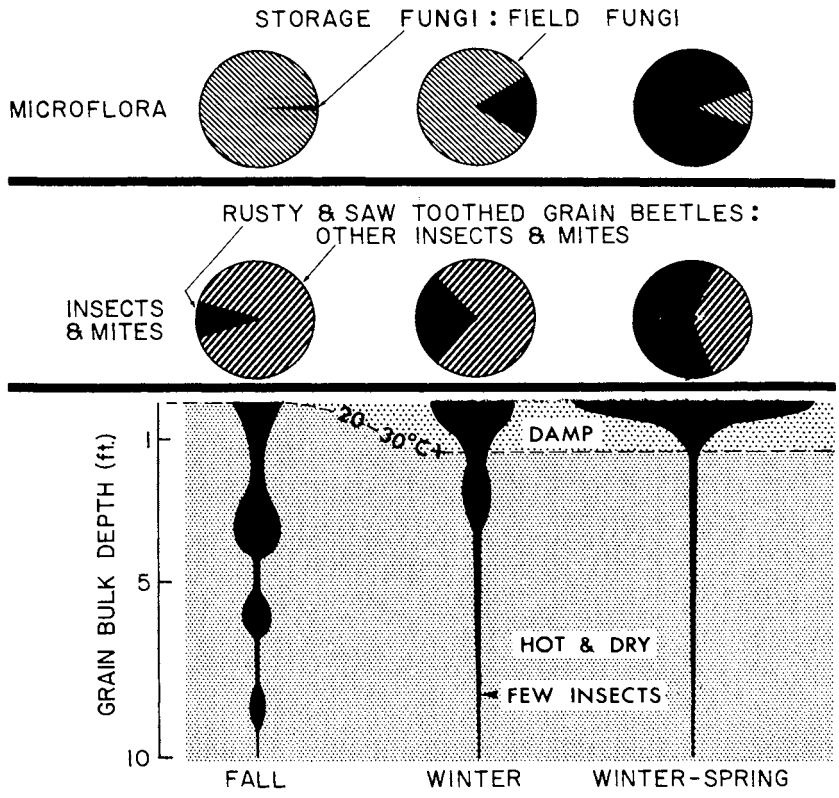

STORAGE FUNGI : FIELD FUNGI
MICROFLORA
RUSTY & SAW TOOTHED GRAIN BEETLES :
OTHER INSECTS & MITES
INSECTS
& MITES
GRAIN BULK DEPTH (ft.)
1
5
10
20-30°C+
DAMP
HOT & DRY
FEW INSECTS
FALL
WINTER
WINTER-SPRING

Fig. 4

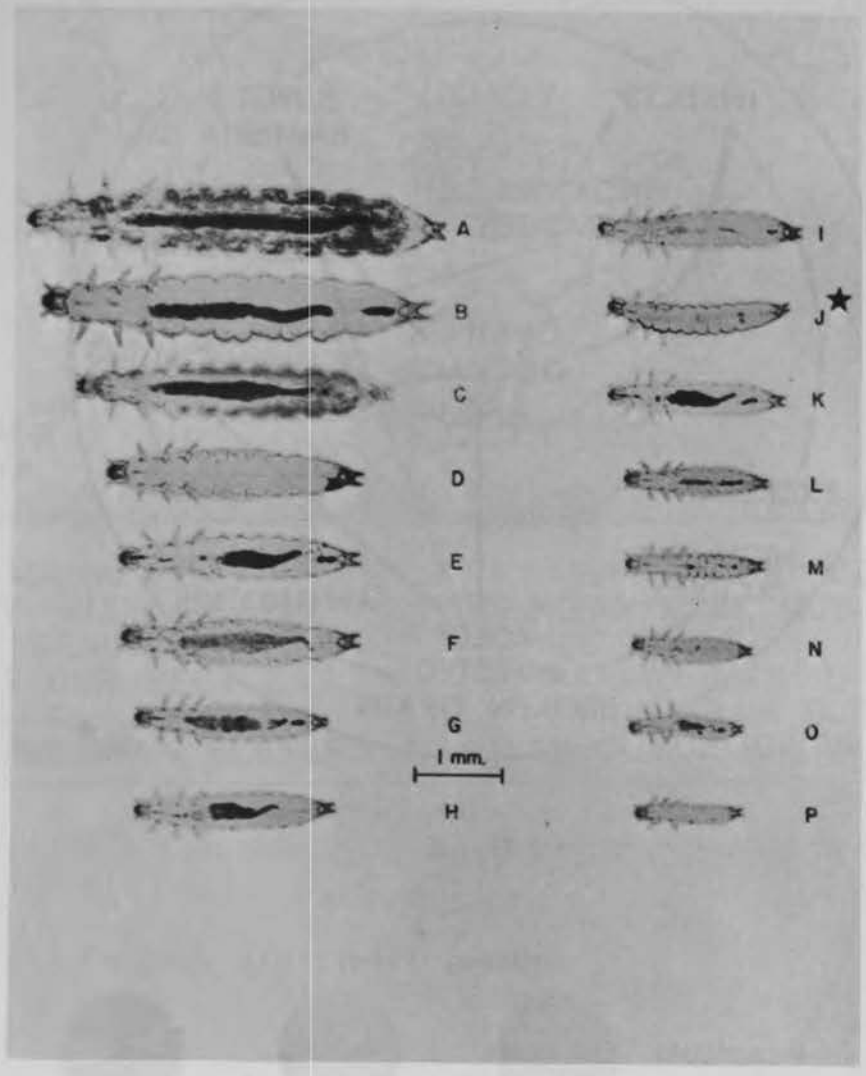


Fig. 5

GENERAL RELATIONSHIPS BETWEEN MICROORGANISMS AND INSECTS

Marion A. Brooks
Associate Professor
Department of Entomology, Fisheries, and Wildlife
University of Minnesota

Probably every informed person today knows that man, domestic and wild animals, birds, even pet frogs and turtles, harob microorganisms in a harmless state. That is, the microorganisms, whether bacteria, yeasts, or other forms, do not cause disease or distress in the host because the microorganisms are not pathogenic in the site in which they are found. For instance, the enteric organisms which normally live in the intestine do not cause disease as long as they do not gain access to other parts of the body, or are not transmitted to other parts of the body of another host animal. Or the microorganisms on the skin, fur, or feathers are harmless as long as they do not enter the bloodstream via a cut or scratch in the skin or mouth.

What many people may not know is that insects have similar relationships with microorganisms. Because insects are so small, and their body parts are microscopic, many a person otherwise well educated is nevertheless surprised to learn that insects even have such organs as a heart, eyes and hearing organs, a breathing apparatus, a mouth and intestine. Once we recognize that insects have functional anatomy, it is easy to realize that these minute creatures may also harbor microorganisms in or on these structures. Just as in our own case, the problems of maintaining their external skin or internal membranes as defense-mechanisms against invasion by microorganisms is vital for them.

Before this subject can be pursued further, it is necessary to emphasize several aspects.

First, we must distinguish between microorganisms which are pathogenic to insects and those which are not; and second, we must recognize that the microorganisms which are pathogenic to us (i.e., man and domestic animals) are not ordinarily pathogenic to insects by normally encountered contacts.

Third, we can analyse from the point of view of the part of the insect's body from which the microorganism can be recovered. Finally, we shall see that there is a wide array of benefits which microorganisms confer on insects, and in fact, without their beneficial microbes, the dependent insects cannot live.

Let us now consider the four points listed above. Insects become diseased and die by the thousands, or entire populations of them may be wiped out, by certain viruses. We approve of such epizootics if they occur in a population of forest tent caterpillars, gypsy moth caterpillars, or cotton pests which are defoliating our valuable trees or crops. Numberous researchers are studying ways to deliberately disseminate such virus diseases among pest insects, because as far as we know these viruses do not harm any other form of life. Some of these insect viruses, consisting of nucleic acids, are protected by being surrounded by a little coating of protein. The digestive juices in the insect's in-

testine act on this protein coat to release the virus, which then gets into the blood and internal organs. Other viruses are naked, lacking a protective coating, and are more vulnerable to destruction by sunlight and weathering. In such cases the means of contacting the insects are much more demanding. We need to know more about the survival of the viruses in natural conditions, and the route of infection. We do not know of any portal of entry except the mouth but possibly eggs may be infected.

Another category of insect pathogens are the bacteria; one of which we hear much today is Bacillus thuringiensis and its relatives. These organisms annihilate susceptible insects by one or more modes of attack. Some forms synthesize a minute crystal of a powerful paralytic agent which very abruptly renders the larvae of some species (especially moth caterpillars) incapable of digesting their food and therefore unable to continue feeding. In other species of insects the crystal is ineffective because of the acidity of the intestinal juices, but then a second toxin may be released from the bacteria which causes something like blood poisoning. Current research is centered around analyzing the crystal with the goal of synthesizing it in vitro to use as an insecticide. Even though B. thuringiensis is a close relative to Bacillus anthracis, the causative organism of anthrax, the former organism and its toxins are harmless to man and domestic animals. In some laboratories these products have been mixed with animal feed because they pass through the intestine unchanged and act in the manure to suppress fly populations.

Particular fungi, molds, and yeasts also kill insects if the environmental conditions are favorable; favorable in this case usually meaning adequate moisture for germination of the spores on the insect's body, and crowded populations so that contact between the insects insures spread of the disease. These pathogens usually enter the insect's body by growing through the skin (known as the integument), either some part of the external body wall or possibly through the mouth. Rarely do they germinate in the intestine and penetrate the gut epithelium. Once inside the body, the fungi consume all the vital organs and by the time the observer sees an obvious growth of mold emerging over the surface of an insect it is already dead. Because the humidity is so important in promoting these diseases, we have had less success in using them for deliberate control of pest insects; but they are extremely effective sometimes naturally. As you will learn later in this symposium, the moisture of the environment in grain storage facilities plays a vital role in the relationship between grain insects and grain mold.

The fourth kind of insect pathogen is represented by one-celled animal-like organisms, much larger than bacteria, called microsporidia. These organisms are taken up with food, but they grow slowly in the intestine and eventually penetrate into other parts of the body and the reproductive organs from where they are transmitted to the offspring. An insect with a large number of these parasites in its body wastes away, especially during winter months.

Whereas all of the above pathogens are harmless to man and other animals, and are potentially useful to us in controlling undesirable insects, unfortunately they do not discriminate between the insects we dislike and the ones we value. They also decimate honey bees, predatory insects, or laboratory insects which we rear for experimental purposes. (There is need here for the development of therapeutics in insect pathology.)

Now we come to the microorganisms which are pathogenic to man but harmless to insects. The obvious criterion here is that a human pathogen must be available somehow to a free-living insect, or the pathogen must be taken up in a blood-meal or from other loci of infection by parasitic arthropods (such as mosquitoes, lice, or ticks, etc.) I shall not discuss the medical ramifications here of arthropod-vectored diseases. The point I want to make is that by casual contact an otherwise harmless insect such as a cockroach, or fly, or wasp, or beetle, may walk through human or poultry excrement, sores on cattle, or decaying vegetation. Then these same insects mechanically transport the microorganisms on their feet or mouthparts to the next visiting place, which could well be a cream pie, poultry feed, or a cut on the face of a calf. We call such insect agents mechanical vectors, because no growth or multiplication occurs in the body of the insect, it is unharmed by the experience, and no biological advantage accrues to the microorganism, other than its transportation and inoculation onto a new substrate. But that latter aspect may have very serious consequences if the microorganism happens to be one of the Salmonella, Escherichia, Erwinia, Staphylococcus, Shigella, Klebsiella, Penicillium, etc., which can cause disease or decay in suitable substrates.

Interestingly, the vectors are never affected by these organisms. Even such virulent organisms as the causative agents of diphtheria, or poliomyelitis, have been fed and injected into some insects (notably cockroaches), and the insects were unaffected by it. The specific examples are too numerous to relate here, but in general one of two processes may occur: 1) either the microorganism is unaffected in these immune insects, it may be recovered, and it is still virulent; or 2) the microorganism is destroyed in the intestine or blood of the immune insect, even though the insect has had no prior exposure, such as vaccination. We call the latter case phylogenetic immunity, or nonspecific immunity, meaning that some factor innately present in the insect renders the microorganism innocuous. But we cannot find any principle in these insects comparable to the antibodies we can demonstrate in higher animals.

Our attention may finally be directed to all those benefits which accrue to insects by virtue of their association with microorganisms. This topic certainly receives far less attention and consequently is even more poorly known by entomologists and the public in general than that which we have just covered. There are four major ways in which insects are benefited. The first and simplest is by substrate modification. This refers to the proteolytic action or other decomposing action of bacteria and fungi, which reduces solid foods to a fluid state or at least partially breaks down their texture so that they are easily imbibed by the insects. Accessory to this is the aroma emitted by fermenting materials which attracts insects to feed. Another benefit is the direct use of the yeasts especially, or algae, or other minute organisms, as food particles. This is practiced by larvae of mosquitoes, fruit flies and others which live in their food. Some beetles, ants, and termites cultivate fungus gardens, feeding on fruiting bodies with seemingly great deliberation.

A considerable number of species of beetles and some plant-sucking and blood-sucking bugs have evolved a very close relationship between particular bacteria and yeasts, to the extent that special little pockets or pits in the lining of the intestine always shelter these microorganisms. Evidently the microbes synthesize an excess of certain vitamins which enrich the diet of the insects, since some of them live on such peculiar and nu-

tritionally inadequate things as wood, tobacco, or paper. Even the method of infection of these insects leaves nothing to chance. Some live microorganisms always are smeared on the shells of the insect eggs, so that the larvae become infected in the process of hatching.

Most elaborate of all is the dependence of other insects on microorganisms which constantly reside inside of cells of the internal organs. Hundreds of species of insects are known to have such arrangements, but assuredly there must be many more thousands which have never been examined. The microorganisms have not been identified and named, because they resist culture and so cannot be studied.

A few experiments have succeeded in demonstrating that some antibiotics will eliminate these mysterious microorganisms, causing a lingering death of the insects thus deprived. So we know that the insects are dependent on their little guests. Precise anatomical modifications provide for passage of these symbiotes, as they are called, to the egg and thus to the next generation, since life without them is impossible. Presumably the symbiotes have been literally a part of the insect's body for so long that they have taken over certain steps in metabolism.

I have tried to survey the general relationship of insects and microorganisms, showing that the consequences to insects range all the way from highly contagious and lethal diseases, through random contact and feeding, on to an absolute dependence upon the microorganisms as a way of life.

The speakers who follow me will tell you about the nuisance caused us by the ability of insects to transport disease and decay organisms into our food and feed.

RESEARCH ON THE BIOLOGICAL CONTAMINATION OF STORED PRODUCE IN ENGLAND

R. W. Howe, Professor
Pest Infestation Laboratory, Slough, England
and Visiting Professor
Department of Entomology, University of Wisconsin

The subjects covered by the title of this symposium form only a relatively small part of the field of stored products and hence cover only a fraction of the work done in the United Kingdom on the subject. I should, therefore, start by describing the organization of our work in this field and try to interpret the attitudes that determine which problems we study. Altogether there are three Government laboratories concerned with stored products, the Pest Infestation Laboratory (PIL) on the Hurworth site at Slough, the Tropical Stored Products Centre (TSPC) of the Ministry of Overseas Development which is on the same site as PIL, and the Infestation Control Laboratory (ICL) of the Ministry of Agriculture, Fisheries and Food, which is at Tolworth in Surrey. There are no hard demarcation lines between the research responsibilities of the three laboratories. PIL mainly undertakes long range research and cannot rapidly change its line of work, so each of the others carries out similar work when they need a quick answer and PIL has no staff available for the project. TSPC deals with the problems of the underdeveloped nations and always has several research workers serving overseas, and ICL is concerned with imported produce and the immediate practical problems of storing it, and with the problems of home grown produce on farms.

All three laboratories have a common origin, the Field Station of the Imperial College of Science and Technology set up more than forty years ago. Here, under Prof. J. W. Munro, the biology and control of insects attacking stored produce were studied on behalf of various commercial and government marketing agencies such as the Empire Marketing Board and the Australian Dried Fruits Board. About 1937, thanks to the energy and foresight of Prof. Munro, the highly diverse British grain industry combined to help finance a 5-man team under Mr. G. V. B. Herford to study the insect problems of the whole industry, and the survey work was completed in 1939. This team, the Hurworth premises and many of the other staff of the Entomology Department were taken over as PIL in 1940, and the remaining members joined in the research program. Very soon some of the PIL staff who, under Dr. J. A. Freeman were especially concerned with protecting food stocks from insects and rodents split off and eventually developed into ICL. About 1950, following visits by Mr. T. A. Oxley and myself to East and West Africa respectively, a Tropical Department was set up at PIL to concentrate on the storage problems of the tropical parts of the British Commonwealth. This grew rapidly, became independent and is now attached to the Tropical Products Institute.

There is still considerable material loss of stored produce in the tropics where the climate favors pests. In the U.K. on the other hand there is little serious loss except that caused by contamination, but it is the commercial companies marketing branded goods that are most concerned and they, of course, are convinced that most insect contamination arises

in retail premises. Public authorities are much more concerned about the risks of chemical contamination than they are about biological contamination. Our regulations refer to visible contamination and we have none against insect fragments and do not test for them. Our laboratories, however, were concerned about the large numbers of insects in corn used for meal in Africa and found very high fragment counts in the meal. Mr. F. Ashman of TSPC has recently developed with Simon Engineering a portable machine using ninhydrin impregnated paper to detect hidden stages of infestation. Mr. S. W. Pixton of PIL has examined the risks of uric acid contamination but did not find the alarmingly high levels reported by some Indian workers.

When insects in stored produce in the U.K. are obvious, the infested food must be destroyed or diverted to animal feed. The regulations under the Food and Drugs Act are administered by Public Health Authorities who may be satisfied if the infested food is withdrawn from sale. Insects may be 'not of the nature' of the food or if more abundant may render it 'unfit for human consumption'. Imported produce is dealt with by Port Health Authorities who usually require infested produce to be fumigated promptly, but may insist that very badly infested lots be destroyed.

Mycotoxins cause some concern at the ports. All imported oilcakes are tested for aflatoxin and if it is found the proportion of the cake used in animal feed is restricted. Tropical nuts such as peanuts and brazil nuts are checked and if any aflatoxin is found these nuts are used for oil extraction or are re-exported. Our laboratories contributed to the early studies of aflatoxin but no mycotoxins were ever found on samples of wheat or barley infested by fungi and in general it appears that so far only tropical strains of Aspergillus have produced significant amounts of aflatoxin. At this moment Mr. G. A. Gilman of TSPC is in Africa trying to establish whether or not there is a link between liver cancer and the suitability of storage conditions for those fungi that manufacture aflatoxins. In the U.K. itself the part played by fungal spores in causing mycoses, especially of Aspergillus fumigatus and 'farmers lung', is still considered a much more serious problem.

In the U.K. we do not seem to have a salmonella problem and there is no pressure on us for the kind of work done in Wisconsin by Wendell Burkholder. Illness caused by salmonellas is not common but we did get a newspaper headline on a minor page when a group of medical men attending a banquet were afflicted.

I can now turn to the work done. At PIL research is shared between five interlocking departments. Three of these, Biochemistry and the two control departments, Insecticides and Fumigants, do a great deal of work related to residues and chemical contamination but the problems being discussed here are tackled by the other two, Biology and Storage. The Biology Department has carried out a lot of ecological studies of insects and mites in storage premises along with supporting experimental and taxonomic work in the laboratory. ICL and TSPC have also done many studies of this kind; in particular ICL has been studying the lesser mealworm in poultry houses. It is unfortunate that this research has had to be dropped temporarily, for it is very interesting that this species should move into a new habitat in at least two places in the world, the U.S. and the U.K. Ecological laboratory studies were carried out on many fungi by Mr. G. Ayerst while he was in the

Biology Department, but have not been published so far as I know. His successor, Dr. J. H. Clarke has set up a large series of reference cultures of fungi. Research on the interrelations of insects and fungi by Mr. S. T. Hill has mainly been concerned with establishing the need of some insects for the active growth of certain fungi though some antagonistic relationships also seem to exist. There is a group in the Biology Department under Dr. D. H. Burges studying the use of bacteria and other pathogens for insect control and the Insecticide Department has made some studies of protozoal diseases of insects in culture.

The Storage Department is particularly interested in the storage of wheat and barley at high moisture content and in this work have the cooperation of Dr. J. H. Clarke whenever fungi including yeasts develop. It is of interest that one of the most pressing problems facing ICL is the safe level of moisture content for the long-term storage of flour protected by polythene covers or 'outers'. At levels below 13% the mites Acarus siro, Thyreophagus entomophagus, and Goheria fusca do not develop but this moisture content affords no protection against Ephestia kuehniella at British temperatures. Mr . S. W. Pixton of the Storage Department has shown how well wheat will keep at normal moisture content if protected from insects and mites. Two varieties have been kept at 12% for ten years with little serious loss of quality. There has been little loss of germinative power in a bin kept at 4.5°C and only 5-12% loss in that stored at ambient temperatures. The free fatty acid content has doubled at low temperature and quadrupled at normal temperatures, and there has been some loss of sugars especially at low temperatures but the deficiency in baking quality attributable to these causes can be corrected by the addition of fungal amylase and potassium bromate. Most relevant to this symposium, however, is the study by Miss Hyde, Mrs. Thompson and Dr. Clarke on the storage at high moisture content. Work on this project started in 1951 was stopped when it was found that the method was suitable only for grain to be used as animal feed, but interest was revived by the introduction of the "Rowett ration', which contains a high proportion of rolled damp barley. They have used flexible butyl rubber airtight bins. When the moisture content did not exceed 18% and the bins were airtight germination was 90% and the grain was free from taint. When the moisture content exceeded 19% germination was poor and small quantities of grain molded -- about 0.25%. The molds grew at the tops of the bins where there was some free space and local increase of moisture content. Where there were leaks molds developed and kept up the concentration of carbon dioxide to give a false impression that the structure was airtight. Clarke divides the floral succession in moist stored barley into five phases, namely, dominance and disappearance of field microflora, yeasts, storage fungi and finally heating fungi. Clarke has found that the field microflora predominates and storage fungi are scarce in barley of 18% moisture content kept by Mr. N. Burrell at about 5° C by refrigeration.

A brief summary of the work of the Pest Infestation Laboratory is published every year and the information mentioned here is taken from that for 1968 which is in press. Tropical Stored Products Information is published by TSPC.

I must thank the following for permission to mention the information they have furnished: Dr. J. A. Freeman in charge of the Entomology Department at ICL, Dr. D.W. Hall, Director of TSPC, and Dr. E. A. Parkin, Director, and Mr. M. E. Soloman and Miss M. B. Hyde who are respectively in charge of the Biology and Storage Departments of PIL.

CURRENT FDA POLICY ON MICROBIOLOGICAL CONTAMINATION

Joe P. Durham, District Director
U. S. Food and Drug Administration
Minneapolis, Minnesota

I appreciate very much the opportunity to participate in this symposium, and to discuss with you the responsibilities and activities of the Food and Drug Administration as they relate to microbiological contaminants in food and feed. The theme of this symposium is indeed a most timely one. A quick review of the agenda is, in itself, testimony to the many and complex facets of this subject, and to respective roles which industry, government, and the academic community have in solving the problems of biological contamination of our nation's food supply.

I have noted that tomorrow's program is devoted to a detailed and technical examination of some of the more troublesome problems currently facing us, such as Salmonella, molds, and insect contamination. Therefore, I will try to limit my remarks on these problems to a discussion of what makes them of prime concern to the Food and Drug Administration, and what we in FDA are attempting to do about them.

Our responsibility stems basically from enforcement of the Food, Drug, and Cosmetic Act, which among other things, prohibits adulterated foods in interstate commerce. It is a broad Act with broad aims, and in enforcement the FDA gauges the levels of practical, reasonable compliance by what is or is not "good commercial practice."

Three parts of one section of the Act specifically cover the microbiological bases for considering a food adulterated. Section 402(a)(1) states a food is adulterated "if it bears or contains any poisonous or deleterious substance which may render it injurious to health." The presence in food of specific infectious bacteria such as salmonellae, the enterotoxins of staphylococci, or the toxins of Clostridium botulinum is considered adulteration under this provision of the Act. No other supporting evidence is needed for regulatory action.

Section 402 (a)(3) states that a food is adulterated "if it consists in whole or in part of any filthy, putrid, or decomposed substance, or if it is otherwise unfit for food." It is under this provision that a food may be found to be adulterated with filth if it contains Escherichia coli, or excessive coliform bacteria, or excessive numbers of bacteria in general. It is necessary to establish, however, that such findings actually constitute filth. Experience has shown that the best and most reliable way of establishing that a product is adulterated with filth is the finding of the filth in the product, supplemented by evidence of observed insanitary conditions in the factory, thus demonstrating that the product has been handled in an insanitary, filthy manner which resulted in the presence of excessive bacteria.

Presented at the University of Minnesota Symposium on Biological Contamination of Grain and Animal By-Products, Minneapolis, Minnesota, February 17, 1969.

The third pertinent part of the section, 402(a)(4), states that a food is adulterated "if it has been prepared, packed, or held under under insanitary conditions whereby it may have become contaminated with filth, or whereby it may have been rendered injurious to health." Thus, establishment inspection evidence alone showing insanitary practices may constitute a basis for legal action.

Certain other important michrobiological considerations are involved in the section of the Act that covers the use of food additives. Our primary responsibility here is to determine that food additives (as defined) are safe, including safety from a microbiological health hazard, and that they accomplish their intended technical effect. Therefore, proposals submitted to the FDA for use of "food additives" must be supported by data adequate to establish these facts.

Now let me turn to the past three years and consider some of the experiences of the Food and Drug Administration which emphasize the continuing problem and identify today's need for improved sanitation and microbiological control in production of our food supply.

In March 1966, through the epidemiological studies and capabilities of the National Communicable Disease Center and cooperating departments of health, the Salmonella contamination of dry milk came to light. You are all familiar with the finding of S. new brunswick in dry milk. During the surveillance and sampling to determine the origin of the contamination, numerous other non-fat dry milks were found to contain Salmonella. The problem has not been overcome, but improved sanitation and positive pasteurization processes, coupled with microbiological control programs appear to be reducing the incidence of contamination.

In April 1966, FDA encountered Salmonella in thyroid powder imported from Denmark, and within a few weeks, in imported thyroid from Italy, Canada, Argentina, and Uruguay. Expansion of our sampling and surveillance operations soon revealed that Salmonella contamination of other products of animal origin, such as pancreatin, pepsin, liver powder, and similar items, both foreign and domestic, was not a rarity. This problem of contamination still prevails and has resulted in many recalls of domestic products and detentions of import lots.

In the tri-state area of New York, New Jersey, and Pennsylvania, there was a widespread outbreak of salmonellosis over the 1966 Memorial Day weekend. Over 300 persons suffered the classical symptoms of salmonellosis in this outbreak. Smoked fish produced by one New York processor was found contaminated with S. java. This same serotype was isolated from stools of patients as well as from stools of several plant employees. Later it developed that S. java was present in the frozen stock of fish for processing.

This incident was soon followed by discovery of Salmonella in carmine red, the alum lake of cochineal. This came to our attention as a result of an outbreak of S. cubana infections in patients in an Eastern hospital. Epidemiologic investigation disclosed that all of the victims had received capsuled carmine red, administered as an intestinal marker or indicator in connection with gastrointestinal studies. Examination of the hospital stock of carmine red revealed S. cubana. Additional hospital outbreaks were reported from California,

Ohio, and Oregon. The production of the contaminated carmine red was traced to one basic manufacturer. The dye is also widely used as a coloring in foods and drugs, and it soon developed that pink summer coatings for candy containing carmine red were contaminated with S. cubana. We found that viable organisms carried over into the finished candy. Food seasonings, meat extenders, and barbecue sauces containing carmine red were also found contaminated. Numerous recalls of these contaminated foods resulted.

More recently, Salmonella was found in chocolate coatings and in finished cholocate candy in which the coatings were used. We do not know the mechanics of contamination or whether the nature of the process is such that conditions favorable to salmonella growth exist in the processing. The industry is currently conducting research in that area.

Salmonella in dried yeast continues to be a problem. Apparently, a high degree of sanitation must be maintained to control and prevent contamination of the finished product. Contamination of dried yeast has serious public health aspects, including the fact that it is frequently used in feeding formulae in hospitals and institutions.

Emphasis thus far has been placed on Salmonella, for it is a good illustration of recent advances and confirmations in our knowledge of food-borne infections. Salmonellosis prior to World War II was not recognized as a common food-borne infection. In recent years, undoubtedly due to better reporting procedures, improved media and methodology, and greater familiarity with the organism, the National Communicable Disease Center receives reports of about 20,000 isolations from human sources per year. Today, on a nationwide basis, the total number of reported salmonellae food-borne outbreaks are running Staphylococcus a close second.

However, recent reports in the professional and public health literature reflect with increasing frequency the isolation of intestinal organisms other than salmonella to be the causative agent in food-borne outbreaks involving large numbers of persons. I am referring to Shigella infections, Staphylococcal food poisonings, and food-associated hepatitis outbreaks.

I have reviewed these experiences only to emphasize one inescapable conclusion. It is becoming increasingly clear that effective control of the food-borne infections, whether they be bacterial or viral in nature, entails a much higher and more rigid level of food plant sanitation than generally has been practiced or required by industry or by health and regulatory officials.

These facts have changed and may further change FDA's view of what constitutes sanitary conditions. While still maintaining an inspection program based on the illegality of gross filth, FDA is placing increasing emphasis on the bacteriological aspects of food plant sanitation. We are now using bacteriological findings quite extensively as a means to determine whether a food has been prepared, packed or handled under insanitary conditions.

The emphasis in FDA inspections today is with the procedural operations and their relation to sources and routes of contamination. A careful observation is made of every phase of the operation from raw material receipt, handling and storage through the various processing

stages to final product preparation, packaging, storage, and readying for distribution. We are concerned with the operations involving personnel, equipment, handling practices, and time-temperature relationships. In many instances we are using a team approach in which an inspector and a microbiologist combine their special skills for an evaluation of the sanitary conditions prevailing during production. Line samples are collected by a microbiologist or bacteriologically trained inspector at critical steps in the processing operation, with particular note being made of places which appear to afford an opportunity for contamination with or growth of microorganisms.

The individuals employed in the plant are often the original source of bacteriological contamination. Unclean hands pass fecal contamination either directly to the food by contact, or indirectly by way of unclean equipment. FDA inspectors check for the proper location of handwashing facilities. They have found no such facilities in some processing areas, leading to the obvious conclusion that the hands of employees are seldom washed. In many plants they observe employees handling a variety of materials and objects and then returning to the handling of food without washing. They see the grossly contaminated all-purpose wipe cloth used by employees to dry their hands, remove accumulations of products, and wipe table surfaces and utensils.

The complete cleanliness of equipment is of extreme importance in the prevention of bacteriological contamination. Unless cleaned at frequent intervals, pipes, pans and other surfaces with which the food comes in contact accumulate enough organic matter to support the growth of micro-organisms which may then be transferred to the food product. Proper stacking and storage of equipment is also essential in order to insure a clean food supply. Trays and other containers of food ingredients have been noted stacked in pyramid style, allowing dirt on the sides or bottoms of the containers to drop into the product. All to often, equipment is stored on the floor prior to being placed on a table surface with which the food comes in contact.

Careful observation of time and temperature is of immense importance in sanitary inspection. High bacterial counts have been found in many cooked ingredients which were stored at improper temperatures for prolonged periods. One plant held a batch of the base mix for chocolate cream pie for three days at room temperature. In addition to high bacterial counts, there was a visible film of mold growth which had developed on the surface. Products from this mix were seized.

At another plant a chop suey mix was left at room temperature all day. The mix was prepared the preceding afternoon and stored overnight in shallow pans in a refrigerated room. When removed from the refrigerator the mix had an aerobic plate count of 600 organisms per gram. After standing at room temperature until midmorning, the count was 1,500 per gram; by early afternoon, two samples counted 320,000 and 660,000 per gram. The counts of the finished product, with the packages placed in master cartons for blast-freezing, ranged from five million to ten million bacteria per gram. The example illustrates the danger of holding a product of good bacteriological quality for several hours at room temperature and it demonstrates the effects of freezing a number of packages in a master carton. Where several individual packages are placed tightly together in the insulating container of a master carton, hours will pass before the temperature in the package drops to a point inhibiting bacterial growth.

Refrigeration at temperatures above freezing is only a temporary expedient; certain bacteria can and do grow at refrigeration temperatures. In time they attain large numbers and eventually spoil the product. To limit the multiplication of most bacteria it is essential to reduce the temperature of food materials to 40° F. or less, within one or two hours. The rate of cooling of large masses of food in deep containers is too slow to prevent substantial bacterial growth for the several hours the food may remain at incubation temperatures. From this it may be inferred that the defrosting of frozen ingredients should be done as promptly as possible. Slow defrosting at room temperatures permits the outer surface or layers to remain at growth temperatures for bacteria while the interior of the block is still hard frozen.

These are some examples of what food plant sanitation means today--what FDA inspectors look for, and what management must consider in formulating or carrying out their own sanitation program. We shall continue to share our inspectional observations with plant management.

At the close of an inspection, management is provided with a written list of observed conditions which, in the opinion of the FDA inspector may lead to a contaminated product. These observations report on basic fundamentals of hygiene; e.g., equipment where food residues have not been removed during clean-up; cracks in equipment or breaks in belts where food particles may lodge; handling of perishable ingredients at temperatures and for periods of time that will permit multiplication of organisms; improper personnel habits, including lack of handwashing, use of an all-purpose wipe cloth, etc. This written report (Form FD-483) contains the observations of a trained person whose only interest is that the products be prepared under clean, sanitary conditions.

When collected, the bacteriological results from FDA anaylsis of in-plant samples are also furnished to the manufacturer. These findings can and should be studied in relation to the list of observed conditions, and the manufacturer can then evaluate his own operation.

Management is finding that a similar approach of critical self-inspection combined with microbiological control is essential to keep abreast of conditions within their food production plants.

FDA's laboratory examination for evidences of insanitary handling always includes determinations for total count, coliforms, and staphylococci. Examinations for Salmonellae, Shigellae, or other enteric pathogens may be made as circumstances indicate. Each of the sanitation indicators has its uses when considered in the light of operating conditions observed. The total count--or, more properly, the aerobic plate count--serves as a rough guide to plant sanitation and will fluctuate with production processes. Heating or cooking of the product will decrease the count; addition of a raw ingredient will increase the count, etc. The coliform organisms are more directly associated with equipment and employee sanitation. Since these organisms are readily destroyed by moderate heat such as used in processing ingredients, their presence in the finished product tends to indicate recontamination.

One member of the coliform group, E. coli, is widely used as an indicator of possible contamination with pathogenic organisms associated with the gastrointestinal tract. Certain strains of staphylocci produce a toxin which when ingested causes typical food poisoning. Isolation of appreciable numbers of staphylococci from a food product is a matter for concern since the product may be or could readily become a hazard to public health. The importance of salmonella needs no further discussion.

If inspection observations indicate such factors as personal uncleanliness, abuses in time-temperature relationships, or dilatory clean-up procedures, and if these observations are confirmed by bacteriological findings in excess of the level indicative of good commercial practice, then the product is considered illegal on the grounds that it was packed under insanitary conditions. In some industries, we are finding the rather incomprehensible situation where a food is kept substantially microbiologically clean or rendered so by in-plant treatment, only to allow it to become recontaminated through neglect or abuse. This kind of a picture is rather dramatic with some series of in-line samples.

It may be well at this point to discuss what we believe to be the three essential elements of an effective sanitary control program for the food processor. These will be discussed from the viewpoint of FDA; however, the analogy relative to industry quality control programs should be evident.

Standards. The first essential is the establishment of standards - the requirements which must be satisfied relative to all aspects of sanitation including methods of evaluation. Standards must be meaningful and attainable. Wherever possible a requirement should be based on the need for it in terms of its contribution to improving the overall sanitation of the operation. In this regard, the Food and Drug Administration has published proposed Current Good Manufacturing Practice Regulations for manufacturing, packing, processing and holding of human foods. When finalized, these so-called "umbrella" regulations will set forth standards of sanitary food processing and serve as a guide to effective compliance with Section 402(a)(4) of the Act. Present plans call for establishing a series of appendice regulations for specific food industries beginning with those food products shown to be susceptible to bacterial contamination. We hope the "umbrella" regulations can be published in final form within the next few months, after which we can proceed with developing regulations for specific industries. While there is much discussion of eventual microbiological standards for foods, there can never be a satisfactory substitute for control-at-source. We believe this may be readily accompliahed by including quality control and sanitation programs as basic elements of food-feed production. We have not discounted the possibility of establishing "tolerances" for bacterial loads in some products. However, it is obvious that we presently lack sufficient data on "normal" bacterial content of most products to establish anything more than purely arbitrary levels.

Education. The second essential of effective control is education. Education must be directed at those who can help if they understand. Plant employees must be fully knowledgeable relative to the reasons for the various requirements imposed upon them and of what constitutes satisfactory compliance with the requirement. The objective of FDA's educational program is to provide facts and techniques that will give industry the greatest opportunity to comply with the law. We are using every means available (within our budget) to disseminate this information. Let me give you an example of how industry has responded

to the educational approach in dealing with the critical problem of bacterial contamination. Last year our field districts, with assistance from various units at headquarters, held 35 workshops targeted toward bacterial contamination and 44 workshops on "Food Sanitation." This represents meeting with some 6,000 people representing approximately 3,000 firms.

Enforcement. The third essential is enforcement. Standards on requirements that may be promulgated are meaningless without enforcement. Suggestions and requests, and education relative to requirements often are effective. However, some forceful motivation or incentive action is needed for application when necessary for continual maintenance of an acceptable level of performance. Our enforcement approach is, and will be, to take regulatory action against the flagrant offender, and against the contaminated products of those plants which continue to operate with a demonstrated disregard for recommended sanitation guidelines or good manufacturing practice.

I believe any FDA presentation before a group such as this one would be incomplete without at least a brief review of our policy regarding Salmonella in animal feeds and the mycotoxin problems.

SALMONELLA

As the first action in a program directed toward reducing incidence of Salmonella in animal feeds the FDA issued a Statement of Policy in the Federal Register on March 15, 1967. This statement in essence announced that Salmonella in the basic protein foods of animal origin (including those of marine and poultry origin) constitutes adulteration within the meaning of Section 402(a) of the Federal Food, Drug and Cosmetic Act. Concurrent with the publishing of the Statement of Policy, the Commissioner of Food and Drugs, addressed a letter to the executive officers of eight national industry associations, including the National Renderers Association, explaining the basis for the Statement of Policy and soliciting cooperation and assistance of the industries in developing voluntary compliance. Recognizing the relatively high incidence of Salmonella in these products, we have been working closely with the U. S. Department of Agriculture (Animal Health Division) and cooperating state agencies in combating the problem. I'm sure, most of you are aware of the State-Federal Salmonella Surveillance Program being conducted by the U.S. Department of Agriculture and the states for voluntary control of Salmonella in animal and marine by-products.

After the initiation of this program, there was found to be an overlap in the activities of the Food and Drug Administration, and those of the Animal Health Division (ANH) and its cooperating state agencies. This duplication resulted from the discharge of statutory responsibilities by FDA, ANH and state agencies in regard to animal disease control and the adulteration of food and feeds.

To avoid this duplication of efforts, ANH and FDA reached an agreement for the coordination of their activities. This agreement permits each agency to meet its responsibilities without a duplication of inspections in individual plants.

The essential features of this agreement are:

(1) ANH personnel or personnel of the cooperating state agency will conduct routine inspection and product sampling of all by-product processors including fish meal producers.

(2) Establishments found to be producing a contaminated product will be reinspected by ANH or cooperating state personnel. Management of such establishments will be given recommendations of measures to take, to eliminate or prevent product contamination.

(3) Management will be given a reasonable period of time to take positive steps to eliminate contamination and correct sanitation deficiencies.

(4) In those instances where management makes little or no effort to eliminate contamination and clean up (correct sanitation deficiencies), the ANH Veterinarian in Charge will provide FDA the name and address of such establishments but will notify plant management prior to taking such action. FDA will take such regulatory action against such establishments as we consider necessary to effect correction. Up until now, the U. S. Department of Agriculture has not experienced the necessity for making any referrals to Minneapolis District regarding rendering plants within our jurisdiction.

In the meantime FDA continues to sample and examine imported animal by-product feeds. Foreign products must comply or the shipments will be detained and refused entry unless they are heat treated or otherwise reprocessed to destroy Salmonella. The State Department has alerted those countries engaged in exportation of meat scrap, rendered tankage, fish meal and similar products to the United States to those requirements.

Prior to August of 1966, it was the practice of FDA to permit diversion of rodent or bird contaminated food grains and other cereal products, to animal feed. This procedure had been well established by precedence over the years, but in light of current veterinary medical knowledge, we questioned the propriety of the practice. After careful consideration of recommendations by our scientists, the field districts were advised that the diversion of contaminated food products into animal feed would no longer be authorized where there was a reasonable expectation that the resulting feed would be contaminated with Salmonella. Exceptions were made where filthy food or feedstuffs would be so treated by heat sufficient to kill Salmonella, and, in case of wheat or other grains containing rodent excreta, a suitable heat process was involved or the product was examined bacteriologically and shown not to contain Salmonella.

Upon learning of this change in policy the grain industry raised questions as to the interpretation of this modification in procedure and its ultimate impact upon the feed industry, and if this meant we were going to start sampling feed grains to check for Salmonella contamination. At this time the answer is still no.

It should be emphasized that our position on diversion of rodent contaminated food applies not only to grain, but to all food proceeded against under the Food, Drug, and Cosmetic Act where Salmonella may be involved. In all cases where disposition of the article under

supervision of the Food and Drug Administration is required by a Court Decree, we must certify to the Court that the article is no longer adulterated or misbranded under the Act. We cannot make such certification where the Salmonella question has not been resolved by heat treatment or bacteriological testing.

While no specific regulatory pressures have as yet been placed on animal feed industry, it would be meaningless and futile to encourage the basic marine and animal protein producers to exercise microbiological quality control and ignore subsequent conditions and practices that might result in recontamination. It would be well for all segments of the feed industry to carefully evaluate their respective roles in the battle against Salmonella contamination and develop and improve sanitation standards and practices.

MYCOTOXINS

The beginning of the current emphasis on mycotoxin started in 1960. Scientists were first alerted to the virulence of one variety of mold when moldy peanut meals decimated several poultry flocks in Great Britain. The toxic factor was isolated and found to be a metabolite produced by a specific strain of mold, Aspergillus flavus, and was named "aflatoxin."

While the known facts on mycotoxins as a food problem are, indeed, meager, I believe that based upon present knowledge, the following conclusions are reasonable:

1. Aflatoxin and other mycotoxins are metabolites of common molds that affect many common foods.

2. The most effective control measure is the prevention of mycotoxin formation by proper harvesting, drying and storage of crops. Improvements in grain handling equipment and methods are making it easier to prevent mycotoxin production, but there will still be problems due to weather conditions; the more frequent harvesting of grain at high moisture levels, or other difficulties which will occasionally require the destruction of moldy commodities or their diversion to another use.

3. Once the aflatoxins, or other mycotoxins, are formed it is difficult to get rid of them. It may be possible to develop processing techniques, treatments, or other control measures which will solve this problem. However, much more work is needed to establish the effectiveness and economic feasibility of the various methods proposed.

4. The presence of the aflatoxins in human food, animal feed, and possibly in animal products consumed by man, is a definite hazard, although the full extent of the risk, particularly in regard to carcinogenesis, has not yet been determined. Meanwhile, it would be wise to avoid feeding aflatoxin-containing feedstuffs to lactating or meat animals when the products are intended for human consumption.

5. Less efficient feed utilization, as well as animal disease and death, causing serious economic loss to the producer, can result when moldy feedstuffs are fed to animals.

These facts have caused us to take a new look at the ubiquitous mold, which had historically been considered objectionable in some instances but not a danger to health. They were generally considered agents of decomposition when encountered in foods, unless they had been introduced for some desirable effect. Specific administrative mold tolerances and guidelines for decomposition were developed to deal with mold contamination and FDA regulatory actions were based on the concept that moldy foods were adulterated because they "consist in whole or in part of any filthy, putrid, or decomposed substance."

FDA is now actively engaged in an inspectional and laboratory program designed to eliminate, insofar as possible, the incidence of mycotoxin contamination in foods and feeds, and to compile further information on the nature and scope of the problem.

Surveillance activities will be directed to food manufacturers, warehouses, and related storage facilities, with the objective of following up with the collection of official samples of finished products when the factory or other findings indicate a potential mycotoxin contamination. Particular attention will be given to those products especially susceptible to mold infection and which constitute relatively important elements in the public diet.

The 17 FDA field districts are now involved in this program. Although any food product meeting the above criteria is fair game, the major categories to be attacked, in the approximate order of priority attention are:

1. Peanuts and finished products such as peanut butter
2. Brazil nuts, in shell and shelled
3. Dry millers corn and finished products such as corn meal, corn flakes, etc.
4. Figs and fig paste
5. Almonds and almond paste
6. Cotton seed flour
7. Rice
8. Wine
9. Beer
10. Other commodities suspected of being susceptible to aflatoxin contamination including:
    Barley
    Beans
    Corn (other than dry millers corn)
    Coconut (including copra)
    Cheese (all types)
    Filberts
    Fruit Juices & Nectars (excluding cranberries)
    Green coffee
    Malt

Olives
Pecans
Sorghum
Walnuts
Wheat

Legal sanctions will be invoked where appropriate. The extremely baneful effect of aflatoxin has led FDA to adopt the strict precautionary attitude that the unambiguous detection of aflatoxins in any food or feed stuff is presumptive evidence that the product is adulterated and is an illegal commodity. Through the cooperation of USDA, state regulatory officials and industry and trade associations, we hope that legal actions can be kept to a very low figure.

In conclusion, FDA is convinced that prevention of bacteriological contamination can be achieved by a well designed, conscienciously implemented sanitation program based upon the microbiological control of the entire food processing operation. Through education and regulatory efforts, the high incidence of food poisoning in our country today can and must be decreased.

STATE LAW VIEWS FOOD AND FEED CONTAMINATION

Gleason M. Diser, Supervisor
Section of Feed Control, Division of Agronomy Services
Minnesota Department of Agriculture

Introduction

In keeping with the guidelines of the program for this series of discussions, it is my responsibility to present the views of state control officials regarding the current status and potential future problems of insect and microbiological contamination of food and feed. Since my assignment in the Minnesota Department of Agriculture has to do specifically with control of livestock, pet and poultry feeds being distributed in the state, my comments and observations will be directed primarily in that product area. Our feed control activities are indirectly involved in food quality insofar as the possible carry-over of contamination from the feed or grain product into the finished food that results from the use of such products is concerned.

Laws Regarding Contamination

Generally speaking, officials responsible for regulating contamination in feeds and foods do so under the "adulteration clause" in their Law. Such clauses usually read something like "The product is considered or deemed to be adulterated if it contains any substance that may reduce or injuriously affect its quality." Specifically, the Uniform State Feed Bill states that "a commercial feed . . . shall be deemed to be adulterated . . . if any poisonous, deleterious, or non-nutritive ingredient has been found in sufficient amount to render it injurious to health when fed in accordance with directions for use on the label." All of the State Feed Laws with which I am intimately familiar carry an adulteration clause worded very much along this line. The Minnesota Feed Law states that "a commercial feed . . . shall be deemed to be adulterated if any material is present in sufficient amount to render it injurious to health when fed in accordance with directions for use on the label." Obviously, no person is permitted to distribute an adulterated food or feed under the provisions of any of the laws.

Insect Contamination

The regulatory standards regarding insect contamination and resultant filth in food products are much more strict than those currently established for animal feeds. At the present time, contamination of this type in animal feeds must be quite significant before action is taken against the product. In the past when lots of grain (e.g., wheat, barley) have been found to contain insect contamination, they have been diverted to animal feed use. Such lots have been accepted and used in products that receive significant levels of heat in processing (e.g., canned foods for dogs and cats). However, in view of current information regarding the pathogenicity of microorganisms carried by certain insects that may infest grains and grain-products, it becomes necessary for feed control regulations to be reviewed and made more rigidly specific in this area.

While it is not covered in the subject matter of this conference, I would like to digress and discuss in some detail the chemical contamination of food and feed products that can result indirectly from attempts to control insects in the field, storage areas and processing facilities. We are becoming increasingly concerned with the manner in which pesticides are transported and stored in relation to products intended for consumption by humans and livestock. Recommendations can be and are made under the laws and regulations covering the distribution of economic poisons to secure proper storage and handling of these chemicals. However, since the laws regulating such products are primarily concerned with labeling and registration, I do not know of any instance in which the specific information is detailed in the law or regulations as to the proper transportation, storage and handling of these chemicals.

In the instance of two recent fires in the state, one in an elevator where soybeans were stored and the other in a feed manufacturing plant, contamination from pesticide materials posed a very serious problem in salvage operations. In both cases the chemicals were stored in the immediate areas next to the soybeans and animal feeds with resultant contamination throughout the storage areas when the chemical containers were destroyed by the fire. The soybeans and feed products had to be declared unfit for food or feed purposes because of the chemical contamination. If the chemicals had been stored in a separate area as recommended, the loss from contamination would have been minimized.

There is another significantly potential source of chemical contamination of foods and feeds that results indirectly from the use of materials to control insects and microorganisms in seeds and growing crops.

It has become common practice to treat all grains and seeds to be used for planting purposes. Treatment, as used in this sense, means that the grain or seed "has received an application of a substance or process designed to control or repel certain insects or disease organisms or other pests attacking such grains or seedlings grown therefrom or has received some other treatment to improve its planting value." Seed treatment has become an integral and important part of commercial seed processing.

In the usually normal course of events the seed processor and/or dealer and the farmer often find that they have a larger supply of treated seed on hand than is necessary to meet their requirements. The problem then becomes one of disposing of the treated products in such a way that no loss will be incurred by the person who has the material in his possession.

Unfortunately, in the minds of many people, the logical method for disposal of surplus treated grains and seed is to divert them into channels where they can be manufactured into feeds for livestock and poultry. However, it is against the law, as well as illegal under federal regulations, to sell such products in Minnesota for use in animal feeds. Feeds containing such materials are adulterated under the provisions of the Feed Law and are subject to seizure.

Over the years there have been many instances reported where feed manufacturers encountered the problem of unknowingly purchasing treated grain through normal trade

channels and used it in the formulation of their feeds. Specific instances have been described by the Minnesota Department of Agriculture in which treated grains in large shipments of wheat and barley were being handled through trade channels for processing into feed. In an instance of the shipment of barley, the treated grain, to which a mercurial product had been applied, comprised only three bushels in a 1700-bushel shipment. However, since there are no levels of tolerance for mercury in animal feeds, the entire lot of barley had to be transhipped and reprocessed into seed barley at a substantial financial loss to the dealer. In this instance the dealer was fortunate in that the barley was of such quality that it could be processed for seed purposes and thus disposed of at seed grain prices. However, this might not be the case in every instance since feed barley is not always of such quality that it can be upgraded to seed grade by processing. Germination of the lot must also be taken into consideration.

The three major classes of seed treatments are based on products identified as organic mercurials, non-mercurials and insecticides. The Food and Drug Administration, under the provisions of the Food, Drug and Cosmetic Act, has established tolerance levels for various chemical constituents which may be permitted in grain and seed crops, and in meat, fish and poultry, as well as other raw agricultural commodities. Any sample of grain or seed which contains chemicals beyond the upper limit of these tolerances is subject to condemnation and the possessor thereof is liable to legal action. These tolerances have been established for various of the chemicals commonly used in treatment of grain and seeds. Since these tolerances are listed in parts per million (e.g.,as little as one pound or less in one million pounds of grain) it can be readily seen that extremely small amounts of treated product can serve to illegally contaminate a very large shipment or lot of grain.

Fortunately, the overall number of instances reported are few where treated grains or seeds have been diverted and fed to animals with disastrous results. However, one must consider that the results from feeding a treated material can range from a loss in egg production, together with an increase to a greater-than-normal percentage of soft-shelled eggs, through decreased growth rate, combined with leg and hock disorders, to actual destruction of certain organs in the body with death of the animal as the final result.

Microbiological Contamination

Obviously, there have been millions of words written and spoken regarding the subject of microbiological contamination of foods, feed ingredients and finished feeds. While there are many microbiological agencies that can be responsible for such contamination, Salmonellae is currently the most "popular" one.

Control officials are concerned with this type of contamination in both ingredients and finished products. It is quite apparent that one contaminated ingredient in a formulation will result in a finished product that is contaminated. It has been pointed out that "one ingredient with salmonella organisms may contaminate large quantities of feed, as well as processing equipment and dust, which may cause contamination of subsequent batches of feed."

The avenues of contamination by this microorganism are many and varied. In addition to the human being or animal that is obviously infected with the organism and suffering from a salmonellosis, a wide variety of animals (both domestic and wild), birds, rodents, reptiles and insects may serve as hosts and carriers. Because of the extensive distribution of this species of organisms under normal conditions in the natural environment, it has been said that salmonella can be found in any ingredient or almost anywhere in nature "if you look hard enough." The process of "looking hard enough" will be discussed in detail later.

While almost everyone seems to agree that salmonella-free ingredients and finished products can be manufactured, no one in industry is in a position to guarantee that feed ingredients or finished feeds are free from contamination by this organism at the time they are consumed. It is extremely difficult (impossible?) to avoid incidental contamination of feeds and ingredients by salmonella (and other microorganisms) under practical conditions in industry. In spite of the fact that the basic materials used in the formulation may have been free (or relatively free) of microorganisms at the beginning of the mixing or manufacturing process, the finished product may be contaminated during the course of processing, packaging, storage and/or transportation prior to its being received by the consumer. The person responsible for the control of quality in such products must be in a position to exert proper surveillance at all stages of the environment.

Product contamination by molds and the by-products of their metabolism (aflatoxins) is rapidly coming to the fore. It was recently pointed out that this source of contamination in foods and feeds for animals and man may become as important to consumer health as dietary nutrients. This type of contamination can also be regulated under the adulteration clause of the typical law.

Problems Associated with Regulatory Activities

Under the provisions of the typical feed law that is currently being enforced in most states, an Inspector is not legally authorized to inspect equipment, facilities and procedures directly involved with the production of feeds. He may concern himself only with the facilities and procedures involved in the storage and distribution of the finished feed and with the quality of the finished product itself. However, the commissioning of state personnel under the present cooperative program with the Food and Drug Administration will authorize inspectors to inspect all equipment and facilities involved in the manufacture and distribution of medicated feeds under the provisions of the Federal Food, Drug and Cosmetic Act. The Good Manufacturing Practices Regulations, together with other specific regulations promulgated under the Act, will be very effective in aiding state personnel in their efforts to control insect and microbiological contamination from all sources throughout the entire production and distribution environment.

Inspection personnel require additional and specific training in securing and handling of samples associated with surveillance of insect and microbiological contamination. It is quite obvious that current procedures in this area must be modified to provide samples that are representative of product and/or production conditions. Extra precautions are necessary to insure that samples do not become incidentally contaminated during the sampling procedure and subsequent handling prior to analysis in the laboratory.

Standards and specifications need to be established to serve as guide lines in regulating insect and microbiological contamination at various stages in the production and distribution of food and feed products. At what level of contamination is the adulterant "present in sufficient amount to render it injurious to health?" It seems quite apparent on the basis of current information that products cannot be delivered to the consumer guaranteed to be free of this type of contamination. Consequently, it will probably require the establishment of tolerances to make any regulatory program effective from a practical standpoint. It is possible that any tolerances so established would vary with each phase or area of the environment. The question must also be answered as to the extent to which Industry should be required or may be permitted to discipline themselves in this area of potential adulteration.

Numbers seem to change daily as to the serotypes or species of salmonellae which have been isolated and identified. It appears that the current number is considerably over 1000 and is probably approaching the 2000-mark pretty rapidly. It is my understanding that public health authorities consider all serotypes of salmonellae to be pathogenic, even though only a few species characteristically produce disease symptoms that are noticeably specific in either humans or animals. In the establishment of standards, specifications and tolerances, this brings up the question of the need to establish pathogenicity of the species isolated and identified in a particular sample. It is quite obvious that there are many species of molds that may contaminate foods and feeds and their metabolites may or may not be toxic to the consuming individual. The level of aflatoxin in a sample is not necessarily indicated by the quantity of visible mold growth. While the molds themselves may be readily identified in most instances, it requires specialized equipment and techniques to isolate and evaluate their metabolic by-products. There has apparently been considerable difficulty encountered in the past in the development of accurate biological assay procedures, including physical and chemical treatments involved in the extraction, transfer and identification of the toxicants.

Activites to Date

Admittedly, the lion's share of regulatory action to date in Minnesota against insect and microbiological contamination has been in the area of the production and distribution of foods. During the past nine years the Division of Laboratory Services in the Department has been carrying out a great deal of investigational work on the incidence of salmonella in products intended for use in the manufacture or preparation of human foods. Emphasis primarily has been on dried milk powder, non-fat dried milk, liquid and dried eggs and other egg products. However, personnel in the Division of Agronomy Services are thoroughly cognizant of the seriousness and magnitude of the problem as related to control of quality and wholesomeness in feeds for livestock and poultry.

As early as August 1964 a limited survey was made of the animal by-products and mixed feeds being offered for sale in Minnesota. This survey has been continued at intermittent intervals since that time.

We cooperated with the Animal Health Division of the Agricultural Research Service, USDA, in the survey that was conducted in 1966 to determine the rate of salmonella contamination in feed ingredients and finished feeds. As you know, this survey was limited

to the basic feed mills in 26 states, including Minnesota. We furnished the survey personnel with listings of feed manufacturers in the state, together with information as to the magnitude of the respective operations and comments as to the type of ingredients and feeds manufactured by each. It is interesting to note that the results of our limited survey agree very well with the levels of incidence found as a result of the USDA Survey.

In September 1966 we met and worked with representatives of the rendering industry in Minnesota. As a result of these cooperative meetings a set of Recommended Sanitation Guidelines was developed and made available to the industry. The Guidelines were patterned after those prepared by ARS, USDA for salmonella control in the processing of poultry and animal by-products (ARS 91-47; September, 1964).

Spot-checking of facilities concerned primarily with the storage and distribution of feed ingredients and finished feeds is carried on by inspection personnel. In instances where possibilities for insect and microbiological contamination exist, recommendations are made for correction in line with current sanitation guidelines and practical manufacturing procedures.

Summary

It is quite apparent that the control of insect and microbiological contamination, including metabolic by-products from these contaminating agencies, in livestock and poultry feeds will continue to assume a position of ever-increasing importance in animal health. Regulatory personnel in the Minnesota Department of Agriculture are well acquainted with the magnitude and seriousness of the problem and limited surveys have been made to determine the extent of contamination in feed products. Certain of the problems involved in carrying out an adequate program of surveillance and control have been evaluated and discussed in some detail. Inspection activities have been carried out and will be intensified and extended in the future with regard to all facilities associated with the manufacture and distribution of animal feeds in our state.

Many of our leading feed manufacturers have found that carrying on a strict quality control program in all phases of their processing and distribution will serve to reduce the levels of contamination in their finished products. The elimination of these areas of contamination will require an educational program as well as a regulatory one. It will require the full cooperation of management and employees in the areas of manufacturing and/or processing, storage, transportation and other activities involved in distribution of the ultimate consumer. Even the consumer will be required to carry out measures in his specific area to insure that the products are kept free from contamination until they are consumed.

It is our intent to continue to appraise realistically each instance or situation where contamination could or does occur and take such measures as practical to control this area of adulteration in the production and distribution of animal feeds.

THE FOOD INDUSTRY'S RESPONSIBILITIES IN BIOLOGICAL CONTAMINATION

E. M. Foster
Director, Food Research Institute
University of Wisconsin
Madison, Wisconsin

Before discussing responsibilities let us define the scope of our topic. I shall restrict the term biological contamination to its public health sense, meaning those microorganisms or their toxic products that may be harmful to the consumer. In fact, most of my remarks will be directed toward contamination of foods with salmonellae.

In contrast, I shall use the term food industry in its broadest sense, meaning collectively those organizations and individuals who are in the business of supplying food to others. As I see it, these comprise three basic groups:

1. Production of basic raw materials on the farm, such as meat animals, eggs, milk, cereals, fruits, vegetables, etc. This category also would include the harvesting of fish and other seafoods.

2. Processing, or conversion of the basic commodity into a form to be used by the consumer. This phase includes slaughtering, milling, dehydration, freezing, heating, curing, pickling, packaging and similar activities.

3. Preparation and service, which includes the activities of restaurants, hotels, catering establishments, delicatessens, institutional kitchens, vending machine operators and any others who prepare and serve food to the public.

According to a recent issue of FORTUNE MAGAZINE the food processing industry is a 78 billion dollar a year business. Similarly, a USDA study revealed that the food service industry grosses more than 22 billion dollars annually. Thus food processing and food service combined, and apart from food production on the farm, amount to $100,000,000,000 annually, or by far the largest industry in the U. S.

What, then, is the responsibility of this giant industry with more than 25,000 firms in processing alone in respect to biological contamination? Its prime responsibility is to deliver safe and wholesome foods to the consumer; i.e., foods that will not make him sick when he eats them. Stated another way, it is the food industry's responsibility to protect its consumers from being harmed by biological agents associated with its products.

Perhaps this statement fulfills my original charge and I could stop at this point; but merely fixing the responsibility is not enough. The matter is not that simple.

Obviously, no one in the food industry wants to harm the consumer any more than the consumer wants to be harmed. Clearly it is to industry's advantage not to hurt its customers. Distributing a hazardous product is wrong on moral or humanitarian grounds; in most places

it is illegal; and it can bring severe economic repercussions. Having a company's product associated with an outbreak of food poisoning is not good for repeat business, it may result in expensive lawsuits, and it often brings unfavorable publicity that damages the reputation of the company or even a large segment of industry.

Why, then, do consumers still get sick from the foods they eat? Why do an estimated two or three million Americans get food poisoning every year? The answer lies in ignorance;

-- Ignorance of the health hazards associated with foods and how to avoid them;

-- Ignorance on the part of the farmer who feeds heavily contaminated meat scraps that cause salmonellosis in his turkey flock;

-- Ignorance on the part of the butcher who spreads Salmonella from one turkey to another during slaughter;

-- Ignorance on the part of the housewife who does not realize that she may transfer Salmonella from a raw turkey to the cooked bird.

The dictionary defines ignorance as "lack of knowledge; unawareness."

Part of the ignorance about food-borne disease does, indeed, accurately reflect a lack of knowledge. When two people died of botulism here in Minneapolis 9 years ago no one had the slightest idea that fish from the Great Lakes might carry C. botulinum. We know it now from our research of the past five years. Until British scientists investigated the death of thousands of turkey poults about 10 years ago no one suspected the existence of aflatoxins.

Most of our ignorance, though, represents a lack of awareness of (or unwillingness to use) information that is already available. We know quite a bit about the public health hazards associated with foods. At the Food Research Institute we have about 75 scientists working on the major food-borne disease problems. Similar investigations are under way at other universities, in several government agencies, and even in a few industry laboratories. But far too many people in the food processing and food service industries are completely unaware that microbiological problems exist; and many of those who do know prefer to spend their time on more rewarding pursuits than worrying about what could happen if things don't go right.

Thus the food industry faces two major tasks in assuring a safe and wholesome food supply for the American public.

1. A great deal more research must be done on the recognition and understanding of food-borne disease agents; and

2. A massive educational campaign is needed to inform everyone from the farmer through the processor and distributor to the restaurant employee and the housewife about safe foodhandling practices. The vast majority of food poisoning incidents recognized by public health authorities can be attributed directly to

faulty food handling. But food processors make mistakes too, and when they do the number of victims can be large. Thus it is incumbent on each processor insofar as possible to assure the safety of his products even if they are abused, as is always likely to happen at some time during distribution, marketing and preparation.

I shall not attempt to justify the needs for research and education. I fully realize that food industry executives DO NOT like to spend money on "defensive" research. New information about microbiological safety will not earn a nickel; at best it can only avoid loss. Likewise, I understand the reluctance of industry even to hint to the housewife that special care in handling is important to avoid danger. No one wants to weaken her confidence in their product. Yet is it right to sell her a potentially dangerous product without at the same time warning her how to handle it?

Now let us turn our attention more specifically to the problem of Salmonella, which has proved to be one of the more difficult food-associated hazards to control. Since other speakers will consider the problem in relation to animal feeds, meats and poultry, I shall emphasize the relation of Salmonella to other food products.

First, let us consider something about the organisms and the diseases they cause. The salmonellae are commonly considered to be animal pathogens. They are often found in the intestinal tract, but they may also occur in soil, surface waters and various other places in nature. Some varieties affect only one or a few species (e.g., S. typhi causes typhoid fever in man; S. pullorum causes fowl typhoid in chickens), but the vast majority -- over 1300 species or serotypes in all -- can infect a wide variety of animals including man.

The usual manifestation of infection in man is a mild to severe gastroenteritis, with vomiting, diarrhea, nausea, and fever lasting 2 or 3 days on the average; but it can last longer. Most cases are uncomplicated, but sometimes the organisms invade other parts of the body and cause a variety of disease syndromes. The disease is most serious in the very young, the aged and the infirm. Death is not common but it sometimes happens.

Although the first Salmonella was isolated over 80 years ago the organisms were generally considered to be pathogens for animals and were left largely to the veterinarians. Full recognition of their hazard to man came during World War II, when outbreaks of salmonellosis in England were attributed to dehydrated eggs shipped from the U.S. and Canada. Further development of the processed egg industry no doubt contributed to the dissemination of Salmonella by eggs. After all, one bad egg can contaminate dozens of sound eggs when they are broken and mixed together.

In the years that followed World War II eggs and egg products continued to be responsible for numerous outbreaks of salmonellosis in humans. Some of these were well publicized, such as an S. thompson outbreak in Canada caused by a prepared cake mix containing dried eggs; and an S. derby outbreak in several large eastern hospitals caused by shell eggs.

To illustrate the role of eggs in human salmonellosis, during a 54 month period from 1962-67 some 133 outbreaks with 11,121 cases were attributed to specific food vehicles in re-

ports to the Communicable Disease Center. One-third of the outbreaks and 40% of the cases were attributed to eggs and egg products.

Meanwhile, though mandatory pasteurization of processed eggs became effective in 1966, contaminated egg products still appear on the market. During the period April 1967 - April 1968, FDA examined 628 samples of market egg products and found 1 out of 5 contaminated with Salmonella. Whereas I doubt that this ratio exists for all egg products on the market, the fact remains that contaminated eggs and products containing eggs are still being offered for sale.

Earlier we spoke about the contribution of ignorance to food-borne disease. An event in 1968 clearly illustrated the need for education of food processors when an estimated 12,000 cases of salmonellosis were attributed to a frozen dessert served at a number of banquets on the east coast. Unpasteurized egg yolks were used in making the product.

People who ought to know tell me the situation is much better today than it used to be. Whereas at one time it was not unusual to find up to 50,000 Salmonella per gram in dried egg products, now both the incidence and the level of contamination are far lower.

Meanwhile, until about 3 years ago little serious effort was made to prevent or control food-borne salmonellosis in man. The FDA investigated outbreaks when they occurred, and made every effort to remove the offending products from the market, but there was no industry-wide surveillance such as we see today. Hence the full measure of salmonella contamination remained unrealized. Then two outbreaks in 1966, largely among young children, were traced to instant nonfat dry milk. These incidents led to a survey of the dry milk industry, which revealed several more examples of contaminated products on the market. Thereafter the FDA accelerated the pace of its Salmonella surveillance of foods and feeds. Though still placing emphasis on products known to have caused disease, FDA broadened the scope of its surveys to include a wide variety of foodstuffs and particularly those containing ingredients with known contamination potential; for example:

1. The incorporation of dried milk in confections suggested a possible hazard. Investigation has revealed several lots of contaminated candy on the market.

2. An outbreak of salmonellosis in hospital patients was attributed to carmine dye, which is employed as an intestinal marker. It is also used as a food color. Several lots of pink summer candy and other products containing carmine were found to be contaminated with Salmonella.

3. An outbreak of salmonellosis among women in Sweden incriminated thyroid powder. This information led to the investigation of certain animal glandular extracts made in this country, and salmonellae were found in a considerable variety of pharmaceutical products, enzymes, nutritional supplements and even gelatine.

The FDA experience of the past two or three years clearly illustrates the adage "look and ye shall find." Although salmonellae are not everywhere, they have been found frequently enough to electrify the food industry. Nothing in my experience has been more successful

the rehydrated product. But we do know they could become hazardous if the rehydrated product were not consumed promptly or kept properly refrigerated.

Before leaving the subject of Salmonella it might be profitable to review what we know about the sources and control of contamination in processed foods:

1. Eggs -- contamination comes mostly from the exterior of the shell, although the contents maybe contaminated if the shells are cracked or checked. Pasteurization before freezing or drying is the best safeguard, but even then recontamination must be avoided,

2. Dried milk -- we don't actually know how dried milk becomes contaminated. Inadequate pasteurization of the raw milk could allow survival, but this is not likely. Contamination of the powder from unclean equipment surfaces would appear to be the most likely possibility. Presence of water in the environment and in pockets in the equipment may lead to multiplication of salmonellae, thereby increasing the hazard.

3. Coconut, dried yeast, smoked fish -- contamination occurs after processing. Sanitation is the key.

4. Milk chocolate -- contaminated ingredients are the most likely source although contamination from the environment can not be disregarded.

Notwithstanding the fact that human salmonellosis has been traced to dried milk, dried yeast, coconut, carmine, and thyroid powder, the fact is that processed foods and food ingredients -- excepting egg products -- have rarely been involved in human disease. If one examines the reports to CDC between 1962 and 1967, egg products and turkey account for half of all the outbreaks and 80% of all the cases. Other meats and poultry have been incriminated much less often than turkey.

It is a puzzling feature of our American system that places so much emphasis on the absence of salmonellae from processed foods yet allows unrestricted distribution of contaminated turkey, chicken, pork sausage and other animal products. That hazard exists from contaminated meats and poultry, especially turkey, is clearly shown by the statistics given previously.

It is not enough just to shrug our shoulders and say, oh well, meat and poultry are under USDA jurisdiction while other foods are under the FDA. This explanation would not give much solace to the New Jersey housewife who purchased a 23 pound frozen turkey for a family gathering last Thanksgiving. Allegedly she thawed the turkey by holding it overnight in the bottom of her refrigerator. It is not clear when she stuffed the bird, but on Thanksgiving Day she cooked it for 7 hours at 300 F. Eighteen people ate the turkey promptly after cooking, 17 people became ill with S. enteritidis infection, and 2 died -- a 17 year old male and a 56 year old female. Salmonella counts in the leftovers were 29,000/gm in the gravy; 100,000/gm in the turkey meat; and 1,200,000,000/gm in the stuffing.

in directing the attention of industry to the problems of bacterial control.

Why has this happened? The answer is very simple. FDA correctly considers the salmonellae as pathogenic agents for man. As such they are considered to be harmful material and their presence constitutes adulteration. It is illegal to sell adulterated food in interstate commerce. Therefore, food containing salmonellae must be recalled from the market and be reprocessed or destroyed. The number of products withdrawn from the market between July 1, 1966 and April 30, 1968, for Salmonella contamination is shown in Table 1. Obviously I do not know the cost of these recalls to the manufacturers, but some of them reputedly ran into the millions of dollars.

Table 1. Products recalled because of Salmonella contamination. (July 1, 1966 -- April 30, 1968)

Product	No. recalls	No. firms
Animal Glandular products	106	16
Carmine red	6	2
Non-fat dry milk	28	16
Confectionery items	36	7
Dry yeast	7	5
Egg products	9	5
Frozen pies	6	2
Other (5 products)	7	3
Total	205	56

The foregoing figures clearly illustrate the new approach of FDA. No longer content simply to remove from the market a product believed to have caused disease, FDA has been zealously looking for salmonellae in products having a reasonable chance of contamination. And when FDA looks they look thoroughly. In examining a given lot of food the FDA analysts regularly test 10-12 "subsamples" of 25 to 100 grams each. Thus they may culture from 1/2 to 2-1/2 pounds of product, and if Salmonella contamination is clearly demonstrated that is enough to condemn the lot.

Although one can not offer convincing scientific argument against this conclusion, there is, in fact, room to question the danger of some of the products that have been involved. We have examined a recalled lot of milk chocolate, for example, that contained approximately 0.4 Salmonella per 100 grams. That is 2 organisms per pound! It is difficult to believe that this number is hazardous in candy, although they are readily detected by test.

On the other hand small numbers of Salmonella may well be dangerous in some products. About a year ago 18 or 20 people in Canada contracted salmonellosis from the consumption of instant nonfat dry milk. We examined some of the product and found 9 Salmonella per 100 grams. Moreover, when the milk was rehydrated and allowed to stand at room temperature or above, the salmonellae grew rapidly and reached enormous numbers within 24 hours. We have no way to know, of course, if the original 9 salmonellae per 100 grams would cause infection. Even to consume this number one would have to drink a quart of

The housewife in this incident obviously made serious mistakes. The turkey was noticeably undercooked, probably because it was not completely defrosted. Almost certainly, Salmonella multiplied in the stuffing during the so-called cooking period.

Here again we have an example of ignorance. The housewife obviously did not know how to handle a large frozen turkey. One may properly ask, though, is it right to sell a contaminated turkey to this housewife without warning her of the potential danger. Essentially, this is the moral dilemma faced by food processors.

Where, then, do we stand on the control of human salmonellosis? As long as we have a vast reservoir of Salmonella in our domestic animal population we will have Salmonella in our food supply. Eradication of Salmonella from domestic animals simply is not practical without a complete change in our system of rearing animals. Take feed for example. It is often suggested that contaminated meat scraps and other feed supplements are the sources of infection on the farm. If this is true it should be fairly simple to eliminate animal infections merely by sterilizing the feed. As you know, this approach is being tried, and no doubt it will help.

But feed is not the only problem. Birds and rodents can carry Salmonella into the barns and feeding pens. The organisms occur in soil and in surface waters. Spread from one animal to another is facilitated by crowding and poor sanitation. Thus it seems completely impractical to eliminate Salmonella from domestic animals without much more information than we now have. We don't even know how animals become infected; or man either for that matter. Take for example, the S. typhimurium enigma. Regularly about 30% of the Salmonella isolations from man and 20% of those from animals are S. typhimurium. Yet this organism is relatively uncommon among the isolates from processed human food and from animal feed (Table 2.). We might assume that meat and other animal products account for the high incidence of typhimurium infections in man; but how do we explain the discrepancy for animals? Obviously much more research will be necessary before we will know how to control salmonellosis in animals.

Table 2. Relation of Salmonella typhimurium isolates to source.

Source	Number of Isolates	% S. typhimurium
Man	20,040	29
Human foods	923	6
Animals	4,201	18
Animal Feeds	314	3

This is not to suggest that improvements can not be made. Control of infection in breeding flocks and herds, elimination of contaminated feed, prevention of overcrowding, isolation of infected animals, and better sanitation on the farm and in the holding pens are steps that will help to minimize infection in animals.

Even without the eradication of animal salmonellosis a great deal of improvement can be made in our processing techniques. Certain slaughtering practices (such as dehairing of swine, defeathering of poultry, the common chill tank for chicken carcases) help spread

contamination from one carcass to another. These should be improved. Careful attention must be paid to heat treatments intended to kill Salmonella (as in pasteurizing eggs). Better sanitation is necessary to prevent recontamination (as in dried yeast, dried milk, smoked fish, dried eggs, etc). And finally, every precaution must be taken to avoid multiplication of Salmonella in a food processing operation. These organisms require only food and water to grow. Control of moisture is a key part of any dehydration operation.

Finally, I can not leave this subject without again mentioning the necessity for educating the housewife and other food handlers in the essentials of safe food handling practices.

To summarize these remarks, it is clearly not practical to eradicate salmonellae from our domestic animal populations at the present time. More research is needed to find the best solutions. Meanwhile, the situation can be improved considerably by better animal husbandry practices and by careful attention to safe food processing techniques. Most important of all, we must train food handlers in proper techniques of preparing and serving foods.

For its part the food processing industry should understand the potential hazards associated with its products and must take every precaution to avoid steps that might exacerbate the danger. This is especially important where new products, processes, packages and serving methods are being introduced.

THE FEED INDUSTRY'S RESPONSIBILITY IN BIOLOGICAL CONTAMINATION

Henry H. Kaufmann, Manager
Grain Research Laboratory
Cargill Incorporated
Minneapolis, Minnesota

The affluent society in which we live has a definite preference for obtaining its protein from non-plant sources. The feed industry - recognizing this preference - furnishes the "raw material input" to maximize the conversion from plant to animal protein. It is our responsibility to furnish a product that does not cause health problems to beast or man.

In the context of this symposium it would seem that rodents, birds, insects, bacteria and molds - and their products - are of prime concern. We should see to it that the raw materials that enter our plants contain no harmful or pathogenic substances, that our plants are clean and operated in a manner that avoids or minimizes chances for deterioration or gives rise to harmful growth and that our shipping facilities and practices maintain the wholesomeness of our products.

Specifically, we should make it impossible for birds and rodents to enter our plants and promptly eliminate those that do get in. And don't forget a tight roof to keep water out. All incoming ingredients should be monitored for insects, bacteria and moldy conditions - including aflatoxin, which is a product of some molds. It is our responsibility to insist that our suppliers furnish us ingredients that are appropriate and enforce this, where necessary, by refusing to buy from those whose record is poor.

Taking the two areas of main concern today, salmonella and aflatoxin, we can develop some specific procedures to minimize or eliminate problems in the mill. Salmonella needs moisture, temperature and time to multiply. By keeping the plant and its equipment clean, avoid material build-up, keeping our ingredients and products as dry and cool as possible and sweeping up and disposing any spillage promptly we can largely eliminate the salmonella problem. Air interchange between receiving, production and shipping areas should be avoided where possible to eliminate air-borne contamination.
"A Survey To Determine The Salmonella Contamination Rate in Livestock and Poultry Feeds" - J.A.V.M.A., Vol. 151, No. 12, December 15, 1967 - contained the following table.

Incidence of Salmonella in Feed Categories

Source	Grain	Oilseed Meals	Fish Meal	Animal By-Product
Percentage positive for Salmonella	0.66	2.28	4.72	31.07

Putting our effort and money where the odds are best, we should pay particular attention to the fish meal and animal by-products we use; why bring in something that is hard to eliminate and may cost us dearly?

We should encourage our good suppliers to do an even better job of salmonella control to attain our goal of zero contamination. Some, if not all, pelleting operations eliminate all salmonella present - it therefore is even more important to prevent any subsequent recontamination. This includes clean carriers, may they be trucks, railroad cars or in-plant conveyors. AFMA is doing a tremendous job in keeping this problem constantly before the common carriers.

Aflatoxin, a potentially carcinogenic substance produced by some strains of molds under some conditions some of the time, is basically a contaminant for the feed industry. While it could be in almost any ingredient, it is more likely to occur in low-grade grains and oilseed meals. Very few cases are actually on record. I know of no practical way to remove aflatoxin from feed that does not also destroy the nutrient value. Proper storage and housekeeping will avoid its production by molds in the mill, simply by imposing conditions adverse to mold growth - dry, cool surroundings, ingredients and products.

An area largely beyond our control but possibly responsive to an educational program is the handling of our products - be it bulk or bagged - by our customers. Improperly handled or stored feed can be recontaminated. The same factors govern time, temperature and moisture. This educational program would not be entirely unselfish. When a problem does arise at one of our customers, too frequently it is caused by careless or ignorant handling after the product leaves our plants. Some of these cases are difficult and costly to explain and sometimes even harder to prove.

Proper storage and sanitation are just as necessary at the dealer or farm level as they are in our plants, possibly even more so since storage may be prolonged. Spillage around feeders should not be overlooked as a source of trouble. At appropriate temperature and moisture content it is an ideal substrate for bacteria and molds.

A great deal of research is being conducted to obtain a better understanding of all these interrelated problems and to develop methods that will avoid or eliminate them in the first place.

In the meantime, it is our responsibility to use only the best ingredients, monitor their suitability by constant sampling and analysis and apply economic pressure where necessary. We should keep our plants and equipment tight, clean and dry, make sure our storage areas do not give rise to problems and emphasize good housekeeping practices.

We should avoid potential contamination between receiving, storage, production and shipping areas, insist on clean carriers for our ingredients and products, and possibly inform our customers of potential problems inherent in improper handling and storage of our feeds.

POTENTIALLY TOXIC FUNGI IN CEREAL GRAINS AND SEEDS AND THEIR PRODUCTS

C. M. Christensen, Professor,
Department of Plant Pathology
G. H. Nelson, Associate Professor,
College of Veterinary Medicine
Mrs. Fern Bates, Associate Scientist,
College of Veterinary Medicine
R. A. Meronuck, Research Associate,
Department of Plant Pathology

Seeds of wheat, oats, barley, rice and sorghum are likely to be invaded by a variety of field fungi before harvest. The principal fungi involved are Alternaria, Fusarium, and Helminthosproium, and the principal sites of invasion are the hulls, especially the inner surface of the hulls of barley, oats, and rice, the inner side of the outer pericarp layers of wheat, and the embryos of all of these kinds of grain. In "weathered" seeds, or in wheat with blackpoint, there may be relatively large masses of mycelium within the hulls or within the pericarps. Most of this fungus mycelium is removed when seeds are processed into groats or into white flour, but whole grain flour or meal will of course contain the same amount of fungus-invaded tissues as the original seeds.

Isolates of some of these common field fungi are potentially toxic. We have grown 109 isolates of Alternaria, from various sources, in autoclaved moist corn-rice, and have fed this to rats as their sole ration. Of these, 79 isolates, or 72% of the total, were lethal in less than 7 days to both rats to which they were fed. Some of these isolates have been tested repeatedly over a period of 2 years, and have been consistently toxic in every test. Some of the lethal isolates of Alternaria have come from common varieties of wheat and from whole wheat flour bought in local stores.

Of 170 isolates of Fusarium similarly tested, 100, or 59%, have been lethal; others have produced estrogenic symptoms in the animals that consumed them. Only a few isolates of Helminthosporium have been tested in this way, but some of these from oats, have been lethal to rats to which they were fed.

Grains and seeds stored with too high a moisture content may be invaded by storage fungi, principally species of Aspergillus, but also at times Penicillium. Many isolates of a number of species of Aspergillus from grains and from other sources when grown and fed to rats as described above have been lethal. A. flavus, which produces aflatoxins, is by no means the only potentially toxic species in the genus Aspergillus. About 50% of more than 80 isolates of Penicillium that we have tested have been lethal, and there is considerable evidence from the literature that several common species of Penicillium can under some conditions produce rather potent toxins.

Some cereal products may be invaded by fungi during or after manufacture. All of the more than 50 samples of macaroni and spaghetti that we have tested over the past several years

have been moderately to heavily invaded by fungi, principally Aspergillus, especially A. flavus, and Penicillium. The fungi that predominate in macaroni and spaghetti products are not the fungi that predominate in outside air, and it seems highly probable that these products are being invaded by fungi during manufacture. We have encountered occasional loaves of partially baked refrigerated bread rather heavily invaded by fungi. All of the more than 100 samples of black pepper that we have investigated have had a very high population of storage fungi, principally species of Aspergillus, Penicillium, and Scopulariopsis. In many samples one of the predominant fungi has been A. flavus, and of the isolates of A. flavus from black pepper that we have grown as described above and fed to rats or ducklings, from 20 to 40% have been lethal in less than 7 days.

HIGH MOISTURE GRAIN HARVEST AND STORAGE

Harold A. Cloud
Associate Professor and Extension Engineer
Department of Agricultural Engineering
University of Minnesota

The corn belt is well along in a revolution which started some 10 to 12 years ago. This revolution has seen the corn harvesting method gradually shifting from the corn picker-corn crib combination to the field shelling operation. According to a USDA report, in 1967 about 45% of the grain corn harvested in Minnesota was harvested as shelled corn. According to the same report the percentage of field shelled corn in Iowa was about the same as in Minnesota with Illinois and Indiana having a considerably higher percentage field shelled, about 65 and 70% respectively. This field shelled corn is being harvested at moisture contents varying from 20% to 35% with a high percentage in the 27% to 30% range. As a result of the field shelling operation, the corn is being harvested in a condition under which it can be safely stored for only short periods of time. This shift in harvesting methods has created some unique problems in the harvesting, processing and storage of this product.

There are various ways in which this high moisture shelled corn is presently being processed for further use. In many operations where the shelled corn is to be utilized in a livestock feeding program it is being held at this high moisture content in essentially "air-tight" containers in which the corn will undergo the ensiling process. This process requires a minimum moisture content of approximately 23% if it is to be stored in an air tight silo. In many cases it is also being stored in conventional concrete stave silos. In this case the corn should have a minimum moisture content of 25% and should be coarse cracked or rolled before it is put into the silo. The coarse cracking or rolling of the shelled corn helps seal the mass so that the ensiling process will take place properly.

At the present time there is a great deal of interest being generated in the holding of this high moisture corn under reduced temperatures. Studies at Iowa State University have shown that reducing the temperature of high moisture shelled corn from 70° F to 40° F will increase the allowable storage time 7 to 8 times. The major use of this technique at present is to hold high moisture shelled corn for relatively short periods until it can be artifically dried. We are seeing increased interest in the use of this technique to hold high moisture shelled corn for later use in a livestock feeding operation. Many farmers are utilizing this technique in combination with their corn drying operation. If they are "layer" drying in a bin and the weather is reasonably cold towards the end of the harvest season they can hold the last bin of corn without drying by cooling it down. They can then feed it out through the winter ar possibly dry it at a later time if desired. This reduction in temperature of the shelled corn through aeration, in most cases, is being done with natural air at the present time. Investigations are in progress on the use of mechanical refrigeration to produce the air temperatures necessary to reduce the corn temperature to increase its holding time significantly. This would seem to hold considerable promise in many areas of the corn belt. If the air conditions are properly controlled through

blending of outside air and mechanical refrigeration, the high moisture shell corn can be held at these low temperatures and actually very slowly dried under these conditions. This is the process which is commonly referred to as "dehydrofrigidation".

By far the most popular method of processing this high moisture shelled corn is to dry it with heated air rather soon after it has been harvested. There are basically three levels of air temperatures that are used in this drying process. In "layer" drying in a bin, low drying air temperatures are used. In this drying method, the maximum drying air temperature used is approximately 20°F. above the outside air temperature. In "batch-in-bin" drying systems, drying air temperatures of 100°F. to 140°F. are utilized. In most column type and continuous flow drying systems, drying air temperatures of 180°F. to 200°F. are commonly used.

Let us look at some of the problems created by this shift in corn harvesting methods. One of the very significant problems is the mechanical damage that occurs in the harvesting, handling, and drying operation. A very high percentage of the corn that is field shelled today is harvested with the standard cylinder-concave combine. Unless these machines are kept in proper adjustment the mechanical damage that can occur in the harvesting process can be very serious. Many farmers just do not take the pains to properly adjust their combines. Actually there is a need for fine tuning of this adjustment when the harvesting conditions change. Fine adjustments may be required when the farmer moves into a new field where the corn may be a different variety or where the moisture content may be different from that which he was harvesting previously. Many farmers will not take the pains to readjust their combines even when they move from harvesting soybeans to harvesting corn. This lack of inexperience and inattention to proper combine adjustment in many instances is creating an excessive amount of damage to the high moisture corn being harvested at the present time. This damage increases storage problems tremendously and provides a means for further contamination of the corn.

Another phase of the processing operation that contributes significantly to the mechanical damage is the drying process. Some of this damage will occur as basic mechanical damage in the augers and conveyors handling the corn into and out of the dryer. Another source of this damage is the stress cracking which occurs in the rapid drying and rapid cooling processes occuring in the standard high temperature dryers. With the terrific demand for increased capacity of these dryers, the manufacturers are continually striving for higher and higher drying air temperatures. This is an easy way to get increased capacity from a given dryer. This stress cracking of the kernels during the drying process makes the kernels more susceptible to further cracking during the handling of the corn after the drying has been completed. A process called "dryeration" has been developed at Purdue University which significantly reduces the stress cracking which occurs during the high temperature drying process. In this process the corn is removed from the high temperature dryer while it is still hot and transferred to a tempering tank. During the 6-8 hour period that the hot corn is allowed to "steep" in this tempering tank the temperature and moisture gradients within the kernels will tend to equalize. After this 6-8 hour period the corn is then cooled slowly with a very low air flow rate. Tests results at Purdue University on this process have shown a reduction in stress cracking from 44% under the standard high temperature dryer process to 7-1/2% under the dryeration process. Another advantage of this process is the increased drying capacity of a given system because the dryer time is not used for cooling.

One area that I feel has been sadly neglected is aeration of dry corn storage on the farm. Much of the shelled corn that has been dried and stored on the farm in Minnesota is stored in structures that do not have aeration facilities. This has, in many cases, created severe problems of maintaining corn quality. Under the large temperature gradients created within the grain mass during changes in outside temperature, moisture migration will occur within the bin and areas of increased moisture content will develop. Severe deterioration of corn quality can occur as result of this moisture migration. A very simple solution to this problem is to aerate the bin with a very low volume of air. This will maintain the temperature within the bin relatively close to outside temperatures and will eliminate the temperature differences that create this moisture migration. It is my recommendation in all of my extension programs that any grain storage facilities to be built should include aeration equipment for maintaining the quality of this corn over extended periods.

I would now like to discuss a subject which I feel plays a major role in creating shelled corn storage problems. A major percentage of all corn sold in Minnesota today is sold as Grade No. 2 corn. The grade requirements for No. 2 corn are:

1. Minimum test weight of 54 pounds per bushel
2. Maximum crack corn and foreign material of 3%
3. Maximum total damaged kernels of 5%
4. Maximum total heat damage kernels of 0.1%, and
5. Maximum moisture content of 15.5%

It is this 15.5% maximum moisture content allowable for No. 2 corn that I would like to discuss.

Under the marketing structure for shelled corn if a farmer markets corn over 15-1/2% moisture content his price is docked. All corn is bought on the basis of a 56 pound bushel. A common discount schedule for high moisture shelled corn is 2 cents per bushel for every per cent moisture content over 15-1/2%. Now let us look at a farmer marketing shelled corn with a moisture content below 15-1/2%. Now, of course, his corn price is not docked since his moisture content is below 15-1/2%. However, this farmer is still paid on the basis of a 56 pound bushel. Let's see what this farmer is actually selling when he sells shelled corn at 10% and 12% moisture contents. When this farmer sells shelled corn at 10% moisture content, for every 56 pounds (one bushel) he is selling 5.60 pounds of water and 50.40 pounds of dry matter. For every 56 pounds of shelled corn at 12% moisture content he is selling 6.72 pounds of water and 49.28 pounds of dry matter. For every 56 pounds of shelled corn at 15.5% moisture content he is selling 8.68 pounds of water and 47.32 pounds of dry matter. We see that when he sells 10% moisture content shelled corn he is giving away 3.08 pounds of dry matter. When he sells 12% moisture content shelled corn he is giving away 1.96 pounds of dry matter. Looking at it another way he could add water to his 10% or 12% moisture content corn to bring it up to 15-1/2% moisture content and he would receive the same money for every 56 pounds of shelled corn. He would be selling water in this case at the price of No.2. shelled corn. He could add 3.7 pounds of water to every 56 pounds of shelled corn at 19% moisture content and wind up with 59.7 pounds or 1.066 bushels of corn

at 15-1/2% moisture content. He would increase his return by 6.6%. In the case of 12% corn he could increase his return 4.3% by adding water to bring it to 15-1/2% moisture content. It is a serious situation when this farmer sells shelled corn at 10% or 12% moisture content. He is taking a beating. This dilemma is not just restricted to the farmer. The elevator operators are operating under the same situation when they market their corn. What is so serious about this situation? The problem is 15-1/2% moisture content corn is not storable corn. The general recommendations for long term storage of shelled corn is a moisture content between 12 and 13%. The following sentences are quoted from USDA Agricuttural Research Service Special Report 22-56.

"When shelled corn is maintained in storage at 9% moisture content or below, the grain is relatively safe from insect activity. For practical purposes, however, farmers who are trying to control insect activity by over-drying their corn usually dry the grain to around 10 to 11% with good results."

We can see that farmers trying to maintain good quality corn in storage will take a severe financial loss when this corn is sold on the open market.

What is the solution to this problem? I feel the only solution to this problem is a re-organization of the marketing structure whereby shelled corn is purchased on a dry matter basis. In this type of structure there would be the usual discounts on cracked corn and foreign material, etc., as well as a discount on moisture content above some safe storage moisture content. Some safe storage moisture content would have to be decided upon but would probably be in the 10-11% range. It is my feeling that as long as we maintain a marketing structure that carries an economic penalty for the marketing of corn that has been dried to safe storage levels we will be continually faced with a contamination and quality problem.

MYCOTOXINS IN FEEDS AND FOODS

C. J. Mirocha

Associate Professor, Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota 55101.

Mycotoxicoses are diseases of animals and humans caused by toxins produced by fungi that have grown in feeds or foods or in the grain or other ingredients of which the feeds or foods were made and then consumed. The mycotoxins may be acutely lethal or chronic and the effects may be insidious as in cancer induction or else they may have other non-lethal but debilitating effects such as effecting salivation or the sexual cycle. The presence of the fungi or their metabolites in the foodstuffs are usually not apparent unless subjected to analysis. Often, through the processes employed in formulation of the food products, the infecting fungi are killed but usually their toxic metabolites remain.

Mycotoxicology has gained prominence as a science only recently although investigations dealing with toxins from fungi date back many years. Perhaps the oldest and best known case of mycotoxicosis in humans is the poisoning due to ingestion of rye ergot, i.e. sclerotia of Claviceps purpurea. The first recorded case of ergotism occurred in the Rhine valley in the year 857 and the most recent, serious outbreak occurred in southern France in 1951 (3). Alimentary toxic aleukia (ATA) is another mycotoxicosis which was rampant in certain parts of Russia, particularly the war years of World War II, and was associated with grains (wheat, rye, oats, buckwheat, millet) which had overwintered in the field (10). The cause of the disease was thought to be due to a number of fungi, chief of which was Fusarium sporotrichioides. Symptoms in humans included those of leukemia, predisposition to hemorrhage and exhaustion of the bone marrow. F. sporotrichioides is usually known in this country as F. tricinctum and has been implicated in diseases of animals associated with moldy corn in the midwest.

Another mycotoxicosis affecting humans is toxicosis reported by the Japanese to be associated with moldy rice. The toxins are often called yellow rice toxins and the most important among them appears to be islanditoxin produced by the fungus Penicillium islandicum. Islandotoxin can cause severe liver damage and hemorrhage often resulting in death (11, 12).

The mycotoxicosis which gave the greatest impetus to the concerted study of mycotoxicology was aflatoxicosis of 100,000 turkey poults in England in 1960 (1, 14). The condition at first defied diagnosis until the cause was traced to a batch of toxic feed that contained peanuts from Brazil.

The peanuts were infected with Aspergillus flavus which it was found later elaborated several potent toxins called aflatoxins. The latter were later found to rank among the most active carcinogens known to man. Since then, aflatoxin was found in a number of products most notable of which was peanut butter. It has also been found in cottonseed meal, maize, rice, peanuts, and in milk from animals who have been eating contaminated food. In one case, aflatoxins was reported in the milk of a Indian mother who was also nursing her child. Substrates such as rice, wheat, corn, oats, oat straw support excellent aflatoxin production under laboratory conditions. Dr. C. M. Christensen (University of Minnesota) is investigating common black pepper as a source of aflatoxin. Black pepper is heavily infested with A. flavus.

Of immediate concern to farmers is a condition which may develop in cattle or sheep which have eaten second-cutting red clover hay. The primary symptoms of animals ingesting bad lots of this hay is excessive salivation sometimes to the point that salivation can be measured by the pail full. In addition, the animals can develop diarrhea, bloat, stiff joints and sometimes death ensues. Swine, chickens, guinea pigs, rats and mice were also found to be sensitive. The disease is caused by infestation of the forage by the fungus Rhizoctonia leguminicola, the same fungus that causes black-patch of red clover (5). The factor produced by the fungus which causes the excessive salivation has been identified as an alkaloid and is called slaframine.

The task of obtaining wholesome non-toxic feed for our laboratory animals has not been an easy one. For example, our laboratory bio-assay for determining toxicity of various feed constituents of microorganisms calls for the use of young, white rats. Many of our experiments are confounded because the animals used as control often die with internal hemorrhaging. These animals are fed commercial preparations of rodent feed or guinea-pig chow. The problem is eliminated when we use freshly ground, grade no. 1 corn or else a synthetic nutrient mixture developed at MIT in Cambridge which costs more than the price per pound of good steak.

There are perhaps two points of view in determining causality in cases of mycotoxicology. One is that the infecting fungus produces the toxin which causes the disease and the other is that the host may produce the toxigenic substance as a response to infection. Both views are correct. To support the latter, the Japanese have found that sweet potatoes infected with Ceratocystis fimbriata and other fungi will produce a number of phenolic substances among which is ipomeamarone, a terpenoid (16). The Japanese are fond of sweet potatoes and eat them as main vegetables and also feed them to cattle. In the past several decades, death of cattle was sometimes attributed

to bad lots of sweet potatoes; the main cause was thought to be the terpene compounds.

Similarly coumestrol, an estrogenic substance, which appears to occur naturally in alfalfa is produced in greater amounts when the plant is affected by foliar disease causing fungi. Insect damage as that caused by the pea aphid can also stimulate its production. It is suspected that coumestrol may be involved in problems of infertility with sheep in Australia (2, 8).

A loss of over 100 dairy cattle occurred in Japan in 1952 and was attributed to the consumption of feed infested with Penicillium urticae which produces patulin (16). This material is a carcinogen as well as a neurotoxin. Another report of toxicosis of cattle in Japan was traced to feed formulated from malt which contained Aspergillus oryzae var. microsporus. The latter fungus produces a newly described toxic metabolite called maltoryzine (7).

Of the mycotoxicoses, three are of particular importance in the mid-western region of the USA and I will attempt to describe them in more detail. They are aflatoxicoses, mycotoxicoses associated with moldy corn and the estrogenic syndrome in swine.

MYCOTOXICOSES ASSOCIATED WITH MOLDY CORN

Dr. E. B. Smalley of the University of Wisconsin has tried to resolve the complex problem of moldy corn toxicoses into its individual components. The disease is sporadic, of particular importance in the midwestern states and associated primarily with corn which was late in maturing and high in moisture content at the time of the first killing frosts. This corn is usually heavily infected with fungi by spring if stored in the conventional manner in cribs without artificial drying (15).

A variety of symptoms accompany this disease syndrome some of which are general digestive disorders accompanied by diarrhea, which at times may be bloody, reduction in milk yield, unthriftiness, lack of weight gain and general feed refusal. According to Smalley, cases of nervous twitching, leucoencephalomalacia and death, are less common, but occur occasionally. An important portion of the disease syndrome is death of the animals as a result of massive hemorrhaging in the stomach, heart, intestines, lungs, bladder and kidneys. Hemorrhaging is an important widespread symptom common to many mycotoxicoses.

Smalley studied and found many toxin-producing isolates in samples of moldy corn associated with problems of mycotoxicoses. Among these were Fusarium roseum, Trichothecium roseum, Fusarium moniliforme, Nigrospora sp. Epicoccum nigrum, Aspergillus flavus, Papulospora sp. Aspergillus versicolor, Trichoderma lignorum and Fusarium tricinctum. Besides A. flavus, it appeared that F. tricinctum produced the most biologically active metabolite which may be responsible for at least part of the problem. The latter fungus is called F. sporotrichiodes in U.S.S.R. and is thought to be responsible for alimentary toxic aleukia (ATA). Symptoms of ATA in humans are reported to be anemia, increased blood-clotting time, hemorrhages in the skin, nose, mouth, gastrointestinal tract, kidneys and subsequent death.

Of the various isolates of F. tricinctum tested, 3 different mycotoxins were isolated. They are 3-hydroxy-4, 15-diacetoxy-12, 13-epoxy-Δ^9-trichothecene, also 3-hydroxy-4, 15-diacetoxy-8-(3-methylbutyryloxy-12, 13-epoxy-Δ^9-trichothecene called T-2 toxin and a butenolide characterized as 4-acetamido, 4-hydroxy, 2-butenoic acid-' -lactone. Certain isolates of F. roseum produce diacetoxy-scirpenol which is also toxic. There are other toxic metabolites produced by other fungi associated with moldy corn toxicoses. It is not known if these toxins are actually involved in the problem as encountered on the farm because none of these metabolites have ever been found naturally occurring in moldy corn. The procedures of chemical analyses involved in detecting these metabolites in feeds is complex and as yet does not lend itself towards analyses of complex feed mixtures.

In summary, evidence for the involvement of all the trichothecane-type metabolites is circumstantial. Toxin producing isolates have been isolated from moldy corn but only a few isolates have been studied for their toxin-producing potential in corn. It is still necessary to demonstrate these toxins in moldy corn in amounts sufficient to account for the symptoms observed in the laboratory.

AFLATOXICOSES

Some of the early work leading to the description and identification of aflatoxins has already been mentioned in the introduction. They are produced by certain isolates of Aspergillus flavus and hence the name aflatoxins derived from the binomial. Besides A. flavus, which is commonly found in many foods, at least four other species of Aspergillus, namely A. parasiticus, A. ruber and A. wentii have been reported to produce aflatoxins. At least four different species of Penicillium have also been reported to produce these

toxins but some authors doubt the authenticity of these of these reports.

There were four aflatoxins (B1, G1, B2, G2) originally described but now there is a total of eight. Those most recently described are called M1, M2, and B2a and G2a. The code letter M stands for milk because M1 and M2 were first discovered in the milk of lactating animals fed B1. Since then, they have also been found in the urine of sheep and rats fed B1, in moldy peanuts, and is indeed synthesized by certain isolates of A. flavus. M1, M2, B2a, and G2a are almost identical to the parent aflatoxin molecule except for a hydroxy group in either the 2 or 4 position of the furane ring.

Aflatoxin has been reported in a wide variety of products such as barley, Brazil nuts, cassava, cocoa beans, copra, coconut oil cake, peanuts, peanut butter, rice sorghum, soya bean meal, wheat, corn, cottonseed, locust beans, palm kernels, raisins (6). It is difficult at times to isolate and identify aflatoxin in some of these products because of the complex mixture of pigments and other metabolites present in the food sample which often confound analysis. Samples obtained from the farm may be even more difficult to analyze as in the following example. We have encountered cases of suspected mycotoxicity (Turkey X Disease) in young turkey poults where samples of the feed were submitted for analysis. No aflatoxin was found in this sample although numerous colonies of A. flavus were isolated. These same samples were analyzed by adding known amounts of aflatoxins into the feed and these were recovered thus establishing the reliability of our method. Upon subsequent incubation of the feed at a higher moisture content(18%) for 3 days, aflatoxin was readily isolated and demonstrated spectrophotometrically. When this feed was incubated longer, and an attempt at demonstration of aflatoxin was made again, none could be found. We assume that other microorganisms develop and metabolize the aflatoxin components. This illustrates the possibility of aflatoxin production in feed lodged in feed troughs of poultry houses where it becomes moist due to excess water and hence allowing aflatoxin to be produced. This would actually be a microbiological niche found in the poultry house which could contain a high concentration of mycotoxins not found in the sample taken from the feed storage area. The biosynthesis of aflatoxins under such conditions may be cyclic.

The discovery of aflatoxins explained the high incidence of hepatoma in hatchery-raised trout which for many years was considered idiopathic. It was found responsible for deaths among turkey poults, ducklings, chicks and young pheasants. The aflatoxins are also toxic to a wide array of mammals.

The compounds are known to be carcinogenic and teratogenic and hence pose potential public health hazard. They are acutely toxic to most animal species, B1 being most active. The single dose LD50 for most experimental animals is in the range of 0.5-10 mg/Kg body weight. The oral 7 day LD50 in the day-old duckling test based on 50-gm body weight is: B1, 18.2 μg; B2, 84.8 μg; G1, 39.2 μg and G2, 172.5 μg. The most consistant effect at least at the lower concentrations is extensive damage to the liver characterized by proliferation of the bile duct cells. Single dosages of about 15 mg of crude aflatoxin injected into 60-70 lb gilts will result in death with extensive internal hemorrhaging.

Chronic exposure for an extended period of time is a bit more insidious and can result in hepatoma. Experiments with rats have shown that feeding a total of 2.5 mg of aflatoxin evenly distributed over a period of 89 days resulted in hepatomas up to 1 year later. Most investigators believe that aflatoxin is the most active hepatocarcinogen known.

The biological effects of aflatoxin are not simple all or none reactions but rather are modified by presence of or lack of certain nutrients in diets. For example, addition of lysine and arginine sensitizes ducklings to the effects of the toxin (13). In monkeys, test animals given 100 micrograms aflatoxin per day and kept on a low protein diet died within 30 days whereas animals kept on a high protein diet lived and showed no signs of liver lesions after 35 days (9).

Synergistic effects or co-carcinogenecity has been noticed in the development of hepatomas in trout fed aflatoxin B1 at a level of 4 parts per billion. Cycloprenoid fatty acids naturally present in cotton seed meal can increase the incidence of hepatomas induced at 4 ppb from about 50% to nearly 100%. Investigators speculate that the high incidence of liver cirrhosis among some Africans may be due to chronic exposure to aflatoxin in their mold-contaminated porridges or brews (4). There appears to be a sensitization of pyridoxine dificient liver cells to the effect of aflatoxin, a condition quite common among Africans with unbalanced diets.

F-2, ESTROGENIC FACTOR (Zearalenone)

The estrogenic syndrome in swine is primarily associated with an estrogenic metabolite called F-2 or Zearalenone, and is produced by Fusarium graminearum (Gibberella zeae) in stored corn and primarily corn stored in cribs. This fungus is not common in corn at harvest, in Minnesota, but once the corn is stored on the cob in a crib and exposed to the weather, it may be

invaded by this fungus and by a multitude of other fungi, some of which also may be toxic. A period of low temperature, or of alternating moderate and low temperature, is necessary for the production of the toxin.

The estrogenic substance F-2 is also produced by Fusarium moniliforme. The latter species unlike F. graminearum is a common invader of corn plants grown in the field and may occur throughout the plant, including the embryo of the kernel. Grain such as this is stored with the organism present and would depend on favorable conditions, usually adequate moisture, for further development.

The estrogen F-2 as well as a closely related and naturally occurring derivative (F-3) has been found in the following feeds and foods: sow gestation and lactation rations, various sow feed formulations manufactured in Minnesota, uncracked corn stored on farms and elevators, Italian rye grass hay, poultry feed formulations, alfalfa hay, and silage. It has also been found in corn used for human consumption and obtained from various market places in Honduras and Mexico.

The symptoms associated with the feed samples that prompted farmers and others to submit them to the University of Minnesota for analysis were abortion and/or infertility in dairy cattle and sows; vulvovaginitis, prolapse of the vagina and fetal resorbtion in sows; drop in milk production in dairy cattle and drop in egg production in chickens and turkeys.

The estrogenic syndrome in swine involves development of a swollen edematous vulva in females, shrunken testes in young males, enlarged mammary glands in the young of both sexes and possibly abortion in pregnant gilts or sows. In some cases, a prolapse of the vagina and rectum may develop.

Under experimental conditions, six week old gilts fed F-2 developed within 7 days enlarged vulvas, mammae, nipples, and prolapse of the vagina. The uteri of these gilts were greatly enlarged and the ovaries were atrophied. The animals returned to normal after being taken off the F-2 ration.

In another test, four purebred yorkshire gilts, all immunized against hog cholera, erysipelas, and leptospirosis, and which at the start and finish of the test were negative for brucellosis and leptospirosis, were fed as follows: the control gilt received normal sow rations; the others received feed containing respectively 25, 50 and 100 percent corn invaded by an isolate of Fusarium known to produce an estrogenic response in rats. The gilt receiving a ration containing 50% of corn invaded by Fusarium developed an enlarged vulva after four days, and aborted after 21 days. The control gilt weaned a litter of ten pigs, and the three gilts fed different amounts of corn invaded by Fusarium weaned a total of 11, or an average of 3.7 per gilt.

In another test with 65-75 lb. gilts, as little as 1 mg pure, crystalline F-2 per day for 8 days was sufficient to incite pronounced tumefaction of the vulva. This is equivalent to 40 micrograms per kilogram. Those fed crystalline F-2 per os in gelatin capsules at a rate of 5 mg per day developed a pronounced tumefaction of the vulva in 5 days and those receiving 25 mg per day developed tumefaction in 4 days. In some of the test animals, a marked atrophy of the ovaries developed. There was a distinct edema and cellular proliferation in all layers of the uterus. The treated gilts had significant epithelial changes in their cervix, characterized by metaplasia of the mucosal epithelium from a normal double layer of columnar type cells to a stratified squamous cellular layer. The squamous epithelium was 15 cells thick in some cases and very irregular in distribution.

In limited tests with turkey poults, we have found that F-2 incorporated into turkey ration will cause an enlargement of the vent. Actually, there is an invagination of the cloaca, and an enlargement of the cloacal bursa and oviduct. The oviduct developed to about 20 times its normal size.

To date, F-2 has been demonstrated to have an estrogenic effect in guinea pigs, white rats, mice, gilts, and turkeys.

In studies involving the biosynthesis of F-2, we have detected another naturally occurring but as yet unidentified compound, closely related to F-2 in chemical structure, which we call F-3. It appears to be an intermediate in the biosynthesis of F-2 by Fusarium graminearum and is found in small amounts in cultures of this fungus. The material F-3 has been found in numerous samples of feed suspected of causing infertility in dairy cattle in Minnesota as well as feed suspected of being the cause of infertility in swine herds. F-3 is readily extractable from biologic material using the same methods and solvents as with F-2. Its absorption spectrum is identical to that of F-2 except that F-3 lacks an absorption maximum at 314 mu.

Recently, 8 different cases of infertility in sows were reported to the University of Minnesota most of which came from northern Iowa. The symptoms described by the local veterinarians included early farrowing of the sows resulting in small pigs being born, abortion, obviously bred sows losing their underlines, fetal resorbtion, and small litter size. The animals were tested for evidence of leptospirosis or brucellosis but none could be found. The samples submitted included rations formulated on the farm as well as special, commercially prepared gestation and lactation rations and sow cubes. In all cases, F-3 was detected in concentrations ranging from 30 to 186 ppm. In at least one half of the cases, Fusarium moniliforme was also isolated from the

feed mixture. It was suspected that the corn component of the formulation was responsible for the mycotoxicity.

After detection of F-3 and the recognition of its similarity to F-2, we suspected that there may be a family of derivatives of F-2, some of which may have estrogenic activity, and perhaps, be more active than F-2. To test this hypothesis, various constituents of the extract were tested for estrogenic activity using the white rat uterus bioassy. These compounds are produced by Fusarium growing on rice and corn. Estrogenic activity was found in all the constituents of the extract tested, but greatest activity was found in a yellow band corresponding to R_F 0.1. For convenience, the yellow band was resolved into all of its individual components which totalled 5 and these in turn were bioassayed on rats and we found pronounced estrogenic activity in 3 of them. Component number 3 was the most active. Although the latter was present only in trace amounts relative to F-2, it was still more active than F-2. This metabolite was named F-5-3.

The newly found estrogenic material F-5-3, has recently been shown to have a molecular weight of 334. Its absorbtion maxima in the ultraviolet are identical to F-2 (236, 274, 314 mu) and it readily forms a trimethylsilyl ether as does F-2. The retention time of F-5-3 as a trimethylsilyl ether on a gas-liquid chromatography column (S.E. 30, 265^{o}C) is just slightly greater than F-2. Its true estrogenic potential has yet to be fully determined.

In summary, the estrogenic material F-2, to a large extent, can account for the many problems of infertility in swine herds in Minnesota and the surrounding states. There is also some correllation between its presence in feed suspected of causing infertility in dairy cattle. Further, another metabolite called F-3 is produced by the same fungus responsible for infertility in swine.

There is a high correllation between the incidence of this metabolite (F-3) in feed suspected of causing abortion or fetal resorbtion in swine. Although a method for its analysis has been developed, F-3 is labile when purified and has not been identified as yet. Lastly, F-5-3 has been isolated from these same Fusarium cultures and is also active estrogenically. A method for its analysis in feed as yet has not been developed.

LITERATURE CITATIONS

1. Austwick, P. K. and Ayerst, G. 1963. Chem. Ind. (London). pp 55-61.
2. Bickoff, E. M., A. L. Livingston and A. N. Booth. 1960. Arch. of Biochem. and Biophys. 88:262-266.
3. Carefoot, G. L. and E. R. Sprott. 1967. Famine on the Wind. Rand McNally and Co.
4. Ciegler, A. and E. B. Lillehoj. 1968. Mycotoxins. In Adv. Appl. Microbiol. 10:155-219.
5. Crump, M. H., E. B. Smalley, J. N. Henning and R. E. Nichols. 1963. J. Am. Vet. Med. Assoc. 143:996-997.
6. Goldblatt, L. A. 1968. Economic Bot. pp 51-62. Vol. 21
7. Iizuka, H. and M. Iida. 1962. Nature 196:681-682.
8. Loper, G. M. 1968. Crop Science 8:317-319.
9. Madhaven, T. V., K. S. Rao and P. G. Tulpule. 1965. Indian J. Med. Res. 53:985-990.
10. Mayer, C. F. 1953. Military Surgeon. 113:173-189.
11. Miyake, M., M. Saito. T. Tatsuno, M. Tsukioka and Y. Sakai. 1960. Acta Pathologica Japonica 10:75-123.
12. Miyake, M. and M. Saito. 1965. In: "Mycotoxins in Food Stuffs." (G. N. Wogan, ed.) pp 133-146. M.I.T. Press, Cambridge, Massachusetts.
13. Newberne, P. M., G. N. Wogan and A. Hall. 1966. J. Nutr. 90:123-130.
14. Sargeant, K., J. O'Kelly, R. B. A. Carnaghan, and R. Allcroft. 1961. Vet. Record 73:1219-1223.
15. Smalley, E. B., W. F. O. Marasas, F. M. Strong, J. R. Bamburg, R. E. Nichols and N. R. Kosuri. 1969. In: Conference on toxic microorganisms. Joint U.S.-Japan Cooperation on Development and Utilization of Natural Resources, East-West Center, University of Hawaii. Oct. 7-10, 1968.
16. Uritani, I. 1967. Abnormal Substances Produced in Fungus-Contaminated Foodstuffs. J.A.O.A.C. 50:105-114.

THE SALMONELLA PROBLEM IN ANIMAL FEEDS

B. S. Pomeroy, Professor and Head
Robert E. Solee, Research Fellow
Mahesh, C. Kumar, Research Associate
Keith I. Loken, Associate Professor
Department of Veterinary Microbiology and Public Health
College of Veterinary Medicine
University of Minnesota

Salmonellosis of food producing animals is not a new problem. Salmonellae inhabit most warm-blooded and many cold-blooded vertebrates. Salmonellosis may express itself in food producing animals as a clinical disease particularly in young animals resulting in high death losses in isolated instances. The inapparent form of the disease is more common with the animals developing no symptoms and the salmonellae continuing to cycle in the animal population indefinitely because of the contaminated environment.

How meat producing animals become infected: Salmonellae will live in the animals' environment for weeks or months. The common sources of salmonellae are:

1. Feed
2. Water
3. Air
4. Insects (flies, beetles, etc.)
5. Free flying birds (sparrows, pigeons)
6. Other animal hosts (dogs, cats)
7. Rodents - wildlife (rats, mice)
8. Cold-blooded animals (snakes, lizards)
9. Fomites (equipment, vehicles, transports)
10. Man
    a. Clothing, footwear
    b. Carrier - excreta

It is the purpose of this paper to limit the discussion to the problem of animal feeds as a vehicle of salmonellae to food producing animals.

There are many published papers that indicate certain feed ingredients are the primary sources of the contamination of the finished animal feeds. Animal, poultry and marine by-products have been shown to be commonly contaminated with salmonellae and pathogenic Escherichia coli. Additional reports indicate that vegetable protein supplements can be contaminated with salmonellae. (1)

In 1966 a Cooperative State-Federal survey was made to determine the incidence of salmonellae in three finished feeds (cattle, swine and poultry) and in their four major ingredients. This survey was limited to basic feed mills in 26 states. A total of 12,770 samples were collected at 724 feed mills. The percentage positive was as follows:

grain, 0.66%; oilseed meal, 2.28%; fish meal, 4.72%; animal by-products 31.07%; cattle feed, 0.85%; swine feed, 3.13%; poultry feed 5.23%. Approximatedly 40% of the feed mills had salmonella contaminated products. Sixty different salmonella serotypes were isolated. The survey also indicated that the pelleting of swine and poulty feed reduced the level of contamination detected from 6.29% to 0.70%. (2)

A Cooperative State-Federal Rendering Establishment Salmonella program was initiated in 1966 and the preliminary report covering the same 26 states indicated that the finished products from over 50% of the rendering plants were salmonellae contaminated. (3)

A more recent report in 1968 indicated that 95% of the plants producing animal protein for livestock and poultry feeds in the U.S. were participating in the program. Of 14,512 finished product samples cultured 2,278 (15.7%) were positive for salmonellae. At the initial inspection, at least one positive sample was obtained from 295 of 718 plants. (41.1%). Of the 243 plants inspected four times at least one positive sample was obtained from 189 (77.7%) (4).

A recent USDA study (1968) conducted in 5 midwestern feed mills was concerned with primary sources of salmonellae and cross-contamination in feeds. Salmonellae were recovered from 65% of the meat meal samples; 18% of accumulations inside the conveyor tubes; 10% of dust samples on the baggers; 26% of swine concentrate meal; 4% of cattle concentrate meal. Twenty-seven different salmonella serotypes were isolated. The results suggested the organisms did not persist and multiply within the mills but that new serotypes were continually being re-introduced. (5)

A local feed manufacturer (Land O'Lakes) has been monitoring meat meal shipments received at its feed plants since 1960 for salmonellae. The results are as follows:

Year	Carloads Tested	% Positive
1960	49	10.2
1961	114	15.8
1962	156	14.1
1963-64	269	27.5
1965	213	30.99
1966	171	32.75
1967	203	48.27
1968	204	41.66

During 1965, trials were conducted to determine the effect of pelleting on salmonella organisms. Samples of pelleted and granulated formulas containing meat meal were routinely tested for the presence or absence of salmonella organisms. While 28.8% of the incoming shipments of meat meal were positive during this period not a single pelleted and granulated formula was positive for salmonellae. (6)

Beginning in fall of 1966 an intensified study was initiated with animal rendering plants in Minnesota. Seven plants were selected to include variations in types of raw materials processed, methods of processing, level of sanitation and types of by-products produced.

Table 1 - Results of Sanitation Program in Rendering Plant A

Plant A - (not Automated)
Base Line - Prior to Sanitation Program

Environmental samples				Finished product samples			
No. Collections	% Positive	No. Samples	% Positive	No. Collections	% Positive	No. Samples	% Positive
15	100	562	13	15	53	40	35
Transition Period - Initiation of Sanitation Program							
11	37	401	3	29	24	90	13
Established Sanitation Program							
11	18	376	0.6	59	3	163	1.2

Table 2 - Results of Sanitation Program in Rendering Plant B

Plant B (50% Automated)
Phase 1 Press Operation
Base Line - No sanitation

-	-	1746	14	-	-	115	30
Established Sanitation Program							
-	-	2297	2.6	-	-	125	3.2
Phase II Expeller Operation (90% Automated) Base Line - Renovating Period No Sanitation							
36	92	932	18	55	42	182	19
Returning to Sanitation Program							
31	58	308	12	43	39	126	7

Table 3. Plant B.

Salmonella Flora of the Environment and Finished Product Samples

FLIES	RAW PRODUCTS	PROCESSING	FINISHED PRODUCT
.....	.....	S. BINZA	X .....
.....	.....	S. BREDENEY	X .....
.....	.....	S. CERRO	X .....
X	X	S. ORANIENBURG .	X .....
.....	.....	S. ALACHUA	X .....
.....	.....	S. HALMSTAD	X .....
X	X	S. SENFTENBERG ..	X .....
.....	.....	S. DRYPOOL	X .....
X	X	S. TYPHI-MURIUM .	X .....
.....	.....	S. ANATUM	.....
X	X	S. MONTEVIDEO ..	.....
X	.....	S. SAINT PAUL ...	.....
.....	.....	S. DUESSELDORF ..	.....
.....	.....	S. MANHATTAN ..	.....
.....	.....	S. URBANA	.....
.....	.....	S. HEIDELBERG	X .....
.....	.....	S. MELEAGRIDIS ..	.....
.....	.....	S. EIMSBUETTEL ...	.....
.....	S. NEWPORT	.....	S. ORION .....
.....	X	.....	S. THOMASVILLE ...
.....	S. NEW-BRUNSWICK.	.....	.....
X	S. THOMPSON ...	.....	.....
S. ANATUM	.....	.....	.....
S. INFANTIS	.....	.....	.....
S. ILLINOIS	.....	.....	.....
S. MINNESOTA ...	.....	.....	.....
S. NEWINGTON ..	.....	.....	.....
S. WORTHINGTON	.....	.....	.....

X = Same salmonella serotype isolated.

Salmonellae were isolated from 241 (17%) of 1,395 bulk samples. The rate of contamination of products ranged from 3 to 33% in individual plants. Salmonellae were isolated from 359 (19%) of 1,901 environmental swabs taken in plants. Isolations were also made from flies collected in vicinity of the plants. Twenty-eight salmonella serotypes were isolated from the finished products, 23 serotypes from the environment and 16 serotypes from flies. In addition 127 strains of E. coli distributed in 37 0 serotypes were identified. The majority of the E. coli 0 serotypes isolated have been reported to be associated with disease in domestic animals and poultry (7).

Intensive studies have been continued in selecting rendering plants (1966-1969). Examples are used to illustrate the results of a total sampling program including environmental and finished product samples in two rendering plants. Table 1., Plant A. illustrates what may be accomplished by practicing a sanitation program. There was a 95% reduction in the salmonella positive environmental samples and 82% reduction in salmonella positive collections. There was 96% reduction in the salmonella positive finished product samples and 94% reduction in salmonella positive collections.

Table 2, Plant B, illustrates that in the phase I period, when no organized sanitation program was in effect, 30% of the finished product was contaminated. There was an 81% reduction in the salmonella positive environmental samples and 89% reduction in the positive finished product samples when a sanitation program was followed. The plant underwent a remodeling program to convert to an expeller operation. In the remodeling period sanitation programs were relaxed. The salmonella contamination in the finished product returned to a level (19%) that was comparable to the base line established during period when no organized sanitation program was being practiced. The same held true for the environmental samples. Since the remodeling the plant has reestablished a sanitation program resulting in the reduction in contaminated finished product. Table 3 illustrates the salmonella flora of a plant is best accomplished by sampling insect population, raw product, processing and finished product areas as well as the finished product.

Studies of live flies collected in and around rendering plants gave the following results: Inside the processing area, 57% of the collections were positive for salmonellae, inside the warehouse, 73% were positive and 14 serotypes were identified in the flies.

In another study sampling of empty boxcars and trucks were done prior to being loaded with animal by-products at the rendering plants. Samples composed of residue on the floor were collected from 33 boxcars and 5 trucks and 47% were positive for salmonellae and 11 serotypes were identified.

Salmonella Infections in Food Producing Animals

Research work has been done in demonstrating that salmonella contaminated feeds did infect swine, poultry and cattle. (8, 9, 10)

The young turkey is highly susceptible to salmonella serotypes. Since research work at Minnesota has been concerned with salmonella infections in turkeys, the turkey will be selected as a model for further discussion on the role of contaminated feed, environment

and hatchery transmission in the salmonella chain. Over a 30 year period approximately 60 Salmonella serotypes have been isolated from groups of young turkeys submitted to the Veterinary Diagnostic Laboratory from Approximately 1500 field outbreaks. Some of the serotypes have become established in turkey breeder flocks and cycle from breeder flock to the hatchery back to young turkesy. S. typhi-murium, S. heidelberg, S. saint-paul, S. chester and S. enteriditis are examples of such serotypes. Whereas other serotypes are found in young poults but are rarely encountered in breeder flocks. S. anatum, S. senftenberg, S. tennessee are examples of such serotypes.

In the 1967 Annual Salmonella Surveillance Report, 41 Salmonella serotypes were reported isolated from turkeys and 52 serotypes isolated from animal protein; 37 of the 41 serotypes isolated from turkeys were isolated from animal proteins. (11)

A current research study is attempting to determine sources of salmonellae in fryer-roaster turkeys. Two examples will illustrate the importance of hatchery transmissions, contaminated environment and feed. Bacteriological examinations for salmonellae are made at several check points. The building is monitored after cleanup and before the poults are placed in the house. Candle out eggs and cull poults are obtained at the hatchery before the poults are delivered to the farm. A sample of the poults that die during the first 10 days are examined and at monthly intervals environmental samples and fecal swabs of 300 birds are examined until the flock is marketed. At the processing plant fecal swabs and intestinal tracts are collected.

The results of Flock 2 are as follows:

Monitoring of the empty building after cleanup indicated S. saint-paul and S. chester were present in the environment. At the hatchery level S. senftenberg and S. typhimurium were isolated from cull poults and pipped eggs. A sample of the dead poults in the first 10 days was examined and S. senftenberg was isolated. At 4 weeks of age, S. senftenberg and S. saint-paul were isolated from 52 of 300 fecal swabs and from the environment of the building (litter and dust) S. senftenberg, S. saint-paul and S. chester were isolated. At eight weeks 46 isolations were made from 300 fecal swabs with S. chester being isolated along with the other two. The same held for the environmental samples. At twelve weeks 15 isolations were made from 300 swabs with S. senftenberg and S. saint-paul being isolated and from the litter and dust, the same serotypes. At 16 weeks at the processing plant, S. senftenberg and S. saint-paul were isolated from two fecal swabs taken from 300 birds at the time they were hung on the line and three S. senftenberg and two S. chester isolations were made from 5 out of 150 intestinal tracts.

Flock 7 is in the process of being studied. Monitoring of the environment before poults were placed in the building was negative. Candle out eggs, cull poults and pipped eggs revealed Arizona infection at the hatchery level. A sample of the poults that died during the first 10 days revealed 15 isolations of Arizona and S. chester. At 4 weeks of age S. chester was isolated from the environment and S. typhimurium from a feed sample taken from the feed storage bin. At four weeks of age, isolations were made from 24 out of 300 fecal swabs and S. chester, S. typhimurium and Arizona were isolated.

Discussion:

There is concern to reduce Salmonella infections in food producing animals particularly swine, chickens, and turkeys. In the young animals and poultry, salmonellosis may cause severe losses. Mature animals may become carriers. Owing to the high frequency of salmonella infections in domestic animals, foods of animal origin offer a potential hazard to public health.

If progress is going to be made in reducing salmonellae in food producing animals, salmonella free feeds must be made available. Special efforts must be made to minimize Salmonella contaminated animal by-products. Studies indicate that meat meal is a primary source of salmonella contamination for the mills and manufactured feeds. Pelletizing of animal feeds is helpful in reducing salmonellae in the finished product but with constant input of contaminated products, cross contamination of the finished products always exists. Progress has been made in the rendering industry to improve sanitation procedures resulting in the reduction of contaminated animal by-products. However, we are a long way from having salmonella free ingredients available to the feed manufacturer. Until salmonella free feeds are available for livestock and pountry, we are simply spinning our wheels.

It must be recognized in addition to feed as potential source of salmonella there are other sources of salmonellae to food producing animals. The producer must realize that improvement in sanitation programs at the farm level is a must along with salmonella-free feed. This will require rather drastic changes in management practices from production to slaughter.

References:

1. Pomeroy, B.S., Siddiqui, Y., and Grady, Margaret K. Salmonella in Animal Feeds and Feed Ingredients; Proceedings National Conference on Salmonellosis. Page 74-77. March 11-13, 1964, NCDC, Atlanta, Georgia.
2. Allred, J. N., Walker, J. W., Beal, B.C., Jr., and Germaine, F. W. A Survey to Determine the Salmonella Contamination Rate in Livestock and Poultry Feeds. JAVMA, (1967), 151: 1857-1860.
3. Salmonella in Livestock and Poultry. ARS 91-48-5, USDA, 1967.
4. Wilson, S.T., Jr., and Germaine, F.W. Salmonella Program in Rendering Plants. Proceedings of the 72nd Annual Meeting U.S. Livestock Sanitary Ass'n., 1968.
5. Nape, W.F. Recovery of Salmonella from Material in Feed Mills. Proceedings 72nd Annual Meeting U.S. Livestock Sanitary Ass'n. 1968.
6. Crane, F.M., Hanson, M. and Schugel, L., Salmonella in Feedstuffs. Feedstuffs. July 8, 1967, 39: No. 27, p.44.
7. Loken, K.I., Culbert, K.H., Solee R.E., and Pomeroy, B.S. Microbiological Quality of Protein Feed Supplements Produced by Rendering Plants. Applied Microbiology (1968), 16:1002-1005.

8. Williams, L.P., Jr., and Newell, K. W. Sources of Salmonellas in Market Swine. J. Hyg. (1965) 32:97.

9. Gibson, E.A. Diseases of Dairy Cattle. Salmonella Infections in Cattle. J. Dairy Res. (1965) 32:97.

10. Snoeyenbos, G.H., Carlson, V.L., McKie, B.A., and Smyser, C.F. An Epidemiological Study of Salmonellosis of Chickens. Avian Diseases (1967) 11:653.

11. Annual Salmonella Surveillance Report (1967) NCDC-USPHS, Atlanta, Georgia.

THE OCCURENCE OF SALMONELLA IN RED MEATS AND POULTRY MEATS AT THE PROCESSING LEVEL

R. Paul Elliott
Head, Microbiology Group Technical Services Division
USDA., Washington, D.C.

A certain percentage of the animals received for slaughter in Federally inspected plants are Salmonella carriers. In the present stage of meat slaughtering technology, and as long as Salmonella carriers are received for slaughter, we cannot be assured that any lot of raw meat is completely devoid of salmonellae. In September 1967, we developed a policy statement with regard to salmonellae in red meats and poultry meats and their products. This policy recognized, first, that the principal hazard of Salmonellosis from raw meats is by cross contamination to cooked foods in the kitchen. It is obvious that a very high level or a very high incidence of salmonellae in raw meats is a more serious matter than a low level or low incidence. We are gradually collecting data to tell us what constitutes high incidence and high levels. Raw product known to be heavily contaminated will be retained and improvements in sanitation will be required to protect future production. Disposition of retained product will depend upon the facts developed in each instance, but will include destruction of salmonellae. We are using other bacteria which indicate poor sanitation practices as tools to measure the possibility of contamination with pathogens, including Salmonella.

On the other hand, there is no excuse at all for the presence of salmonellae in a ready-to-eat or warm-and-eat food product. These are often not heated enough in the home to destroy salmonellae; therefore, when we find these bacteria in such a product, we will take every action necessary to remove it from the market. This could include seizure through court action or recall by the firm. We will sample and analyze any such seized or recalled products to determine the extent of contamination, and will require appropriate disposition or reprocessing of contaminated foods to destroy these bacteria. Such foods may then be used for human or non-human use as determined by the condition and character of the product. We will make a special study of operating procedures to detect the source of the contamination. On the basis of information obtained, we will require improvements in sanitation.

As time goes on, we will be collecting general information about sanitation in ready-to-eat products. When we detect indicators of insanitation such as Escherichia coli, and trace our findings to poor sanitation, we will consider these ready-to-eat products to be hazardous because of the potential presence of salmonellae or other pathogens. We will, therefore, treat such products as if they did indeed contain Salmonella.

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We have recently completed two major studies on the incidence of Salmonella in domestic and imported meats in the United States. The survey of incidence in domestic meats was for one entire year, starting November 1, 1967, and extending to October 30, 1968. Nearly 11,000 samples were analyzed from firms representative of U. S. production of meats from cattle, swine, goats, sheep, horses, chickens and turkeys. In all, the production of 80 plants was sampled. This is by far the most extensive study of its kind ever conducted. For cattle, swine and chickens, there were 15 plants sampled each week for a year. For turkeys, there were 15 plants sampled each week during the portion of the year in which turkeys are produced. As you may know, there is a period between January and July when very few turkeys are slaughtered. For goat and horse, we sampled all of the firms which regularly produce such meat. For sheep, we sampled 12 firms for about an eight month period. Our choice of firms was made to represent both area and poundage so that we feel safe in concluding that the data on these are indeed representative of U. S. production. In each instance, feces from the cecum were obtained to measure the incidence of Salmonella in animals received for slaughter. In each instance, various suitable parts were obtained to represent the incidence in meats. For example, in chickens, we sampled the feces, and the back, neck, and gizzard. In turkeys, we sampled only the feces, neck and gizzard. In red meats, we sampled the feces, flank and liver. In the case of pork, we also sampled raw sausage.

The final figures for beef showed 1.1% positive for Salmonella, for pork, 7.8% positive. Turkey was a surprisingly low 1.3%, sheep 1.6%, and goat and horse 5.5% and 5.4%, respectively. Chicken parts were 3.9% positive.

During the course of this study, which was contracted to a private laboratory, our Beltsville laboratory made a separate investigation of the production of the same 15 chicken eviscerating firms. We sampled a total of 597 whole chicken carcasses, between September and November 1967, and determined the incidence of Salmonella in the whole carcasses. We found to our surprise that 28.5% were positive for Salmonella, or about 7.5 times the incidence in our major study. However, in this instance, we used the entire bird, weighing about 1200 grams, as opposed to the small portions of 40 to 100 grams which resulted in the 3.9% incidence. The sample size was the obvious reason for this difference, since the laboratories were using identical procedures otherwise.

If the level of salmonellae on positive carcasses is very low, then sample size could explain this difference in incidence. We have information that would indicate the level of Salmonella on positive carcasses to be very low. We made a study of nine additional chicken evisceration plants giving particular attention to the chilling operations. Of the 50 birds found positive for salmonellae, 38 had levels below 30 cells per carcass. We analyzed liter samples of water. About 50% of these samples proved to be positive, but of those that were positive, most contained very low levels of salmonellae. In the one instance in which chlorinated water was used for chilling, salmonellae and the related enteric bacteria were all absent.

We have also done a short-term study to determine whether there might be a difference in incidence of salmonellae in non-federally inspected whole chicken carcasses as opposed

to the federally inspected. We obtained 147 samples which we think represented U. S. production in non-federally inspected whole chicken carcasses. Thirty-four of them were positive, which gave us 23.1% positive for Salmonella. Since this was done on the whole birds, it compares directly with our short term study on whole chicken carcasses from federally inspected plants, 28.5%. The incidence of Salmonella in the non-federally inspected birds seems not to be significantly different from that in the inspected birds in the study.

We also made quantitative studies of these non-federally inspected chickens. Of the 34 positives, 27 were positive in washings representing only the entire bird. Again, this shows that the level of Salmonella in positive carcasses was indeed very low.

We have some small amount of evidence to show that the numbers of cells of Salmonella on pork parts are also very low. For example, our contract laboratory found five positives in 49 samples of pork liver analyzed. Our Beltsville laboratory also found five positives in these same 49 samples, but none of them were the same five. We found furthermore, that when nine samples of pork sausage our own laboratory had found to be positive, were re-analyzed in our laboratory, we found six of them to be negative on the second determination. We concluded that the salmonellae are present in low levels and rather poorly distributed in pork products.

These findings of low levels have since been confirmed by workers at the Massachusetts Institute of Technology, and the Canadian Department of Agriculture. There are, of course, wide differences reported among various investigators as to the incidence of Salmonella in poultry meats and red meats. We can explain such differences in part by the individual sample size, and in part by the laboratory methodology; but there is one other factor which most studies have failed to recognize. Our study on domestic meats was nationwide. We have analyzed the findings by areas and found that the carrier rate of the various animals varies greatly. For example, in chickens, the carrier rate varied from 0.5 to 14.8%. In turkeys, the carrier rate varied from 0 to 10%. In pork, the carrier rate varied from 5% to 33%. Most surveys reported in the literature are conducted in a very limited geographical area. We feel that our results are more representative of U. S. conditions than any reported heretofore because of the national sampling program.

Raw meats now entering the United States are rarely contaminated with Salmonella. None of the cooked and ready-to-eat products imported into the United States were found positive for Salmonella.

Now, let us consider some of the processes within the evisceration plant. The inspectors examine each carcass individually, looking for evidence of disease. Asymptomatic carriers of Salmonella cannot be detected by this means. The inspectors are ever alert to an undue amount of fecal contamination of the meat surfaces. Any such must be cut away with a knife. Washing is not permitted. However, the studies of Mildred Galton and others have shown salmonellae to be spread by several of the procedures now in use in slaughterhouses both for red meat animals and poultry. For example, she found that almost all of pork carcasses leaving the dehairing machine contained salmonellae, al-

though a relatively low percentage of animals entering this machine were positive. In poultry operations, the defeathering machines spread Salmonella from the feces of carrier birds to the surfaces of other birds. In order to demand changes in these very efficient pieces of equipment, we must have alternatives; otherwise, the costs of production would skyrocket. In a similar way, we cannot, at this time, demand that utensils and hands be sanitized between carcasses, because again, this would slow up production to an unwarranted degree. Several investigators have stated that the chill tanks are a source of cross contamination among poultry carcasses. Doubtless, most of the investigations that showed this were made before Federal inspection became mandatory. Under Federal inspection, overflow must equal one half gallon per hour for each bird chilled. Our investigators showed the surprising result that 20.5% of the birds entering the chill tank were positive for Salmonella and 20.5% exiting the chill tank were positive. The general level of enteric bacteria fell because of the washing action of the chill tanks. There was no evidence that the incidence of salmonellae increased.

WHAT TO DO

We cannot destroy the industry because Salmonella is present on raw poultry and meat. To make changes in processing that would prevent all cross contamination between carcasses would introduce costs that would be excessive. We would, of course, be able to reduce the incidence of Salmonella-positive meats by reducing the number of Salmonella carriers received for slaughter. This is a matter primarily for the animal health people. We would like to know what procedures could be used on the meat itself to destroy salmonellae. Chlorine is not particularly effective except at extremely high levels, because it is taken up rapidly by the flesh itself and the salmonellae are protected by layers of fat and by the hair or feather follicles. The iodophors tend to turn the white skin of chickens yellow and the feather follicles blue. Heating tends to destroy the natural fresh bloom of chickens; the carcasses turn white. Perhaps this is not too serious a matter, but it would require the entire industry to change its outlook and the consumer to change his habits. There is an interesting possibility in this for some of the red meats. It has been found that a spray of water at 210° F for a few seconds will destroy most of the flora on the surface of beef. Whereas the beef appears slightly cooked during this process, it soon returns to its natural bloom. The shelf life is extended because of the reduction in bacterial level on the surface. We have not yet determined whether this would also destroy salmonellae, but we see no reason why it might not.

We have been determining the effect of freezing on salmonellae and have found that there is little or no reduction in incidence. Apparently, the fat and protein materials protect salmonellae from death, so that freezing has no effect on the incidence.

There are many sanitation requirements now in effect in federally inspected plants. There is no doubt that these requirements reduce the incidence and level of Salmonella. For example, the hides of beef cattle may be pulled with great care to avoid contaminating the surface of the meat. The gut of red meat animals is carefully tied before it is severed from the carcass. Gross fecal contamination must be cut from meat surfaces, not washed. There are many such requirements. Two new sanitation manuals (for meat and poultry) have been prepared for use by inspectors.

Recently, all limitations on the use of chlorine in poultry plants have been lifted. Whether the copious use of chlorine will reduce Salmonella incidence is hard to say.

The Microbiology Group has expanded considerably in size, so that we now have a total staff of 26. This permits a much more active program of microbiological inspection and laboratory analysis. Nearly all samples received are analysed for Salmonella, many of them quantitatively.

Our Laboratory Branch has started an epidemiology program, to trace back specific outbreaks to determine why red meats or poultry meats were involved in food poisonings. This should reduce outbreaks by correcting some of the causes.

Our laboratories are still working on various aspects of this matter and would welcome your suggestions.

THE LANGUAGE OF GRADES*

Willis E. Anthony, Professor
Department of Agricultural Economics
University of Minnesota

I see your symposium program lists but one economist in two days of presentation. To me this could mean one of three things: The economic issues involved in biological contamination of grain and animal by-products are not very important; the formulae for the resolution of the issues are widely known; or the economic problems of biological contamination are so great they cannot be tackled in a two-day program which also covers the physical properties of biological contamination. I will openly subscribe to the latter interpretation and forge ahead to do in the next half hour what, by definition, cannot be done. If this sounds confusing, it is not entirely by accident. The economic issues of grading are complex. Their resolution usually requires a compromise of conflicting interests. It is a case of "what it would be nice to know" versus "the cost of getting the information." Grading is particularly important in a market economy like the U.S., because people generally do of their own volition what they are paid to do.

Grades as Language

There are economic issues of grading involved with biological contamination of grain and animal by-products. I would like to help you digest your lunch in the next few minutes by talking about the language of grades. And it seems to me that it is useful to think of grades as language. In some respects it is unfortunate that we use the term "grades". The word tends to connote a rank ordering of quality -- much the same as the grading of student examination papers from A through F. "A" is best, "F" is poorest. So in wheat: No. 1 is best, No. 5 is poorest. In beef: "Prime" is best, "Canner" grade is poorest -- right? Wrong! It all depends on the use for the commodity. For example, "Prime" beef is definately not "best" for sausage. Another example: high-moisture corn, which would be "sample" grade, is a more desirable commodity in many feedlots than is No. 2 corn of 15.5 percent moisture. So it is useful to think of grades as a set of descriptive characteristics which are not necessarily a rank ordering of quality. Although grades are often associated with quality rank ordering, they need not be, and probably should not be so conceived. Grading, then, is simply the process of segmenting a highly heterogeneous supply of a commodity into smaller categories that are meaningfully different. The "grades" are the language used to succinctly describe the categories, and the labels attached thereto. In this sense, the assigning of commodities to their primary commodity class is also "grading". When an elevator operator unloads a truckload of grain and spouts it into the oats bin he is assigning it to its primary class, and performing the first grading (if it turns out to be corn, he did a poor grading job). This is all rather elementary. Yet, I think it is necessary that we firmly establish a concept of the purpose of a grading system before we talk about change and modification of a given set of grades to handle a

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current problem, such as biological contamination.

Grades need not be terribly complicated. For example, I am sure you all heard the saying going around some time ago describing the difference in miniskirts. It was said there are three kinds: "Short," "Shorter," and "Wow!" This, I submit, is a grading scheme. Of course, you say it is not a very meaningful set of grades. Those of you who are more serious students of the subject know you need information on additional characteristics to make a complete quality evaluation. Information on the shape of the knee, thickness of the calf, diameter of the ankle, length of the limbs, and so on, would be helpful. Maybe some information on biological contamination would also be welcome. However, just as in grain grading, it may cost more to get some information than the information is worth. A grading system must strike a compromise between the set of all those factors which would be nice to have, and those which it is economically practical to obtain.

Most of you would agree that "short," "shorter," and "wow" is rather a crude grading system. But do you realize that early grain grades were in such ambiguous terms as "dry", "damp," and "unsound." These also seem crude to us now. We have come a long way since then. Or have we? Like many things, I suppose it is all relative.

Development of U.S. Grain Grading

Consider the start of the grain trade. In the early days of grain trading there probably was not too much need for a sophisticated set of characteristics describing categories of a commodity. Remember that almost all grain was processed by the local miller. He was in a position to know the quality of the grain in the surrounding area. He was able to watch it from planting through germination, sprouting, and on to harvest. He probably knew more about the likely milling qualities of any given crop than the man selling it. If he was an alert individual, he could know a great deal about the grain entering his mill. But as we lengthened the market channel from the harvest field to the mill it became more and more difficult for the miller to identify any given lot of grain with its source. And it became even more difficult to know enough about the environment of the source to be able to measure and evaluate the relevant qualities by first-hand information. Thus, the trade adopted some descriptive standards to communicate a few of the essential quality characteristics. More and more criteria were added to the grading system. But communications became confusing as different trade groups and different markets each developed their own grades. Often the same grade name meant different things in different markets. You can imagine the communication problems. Buying grain on grade was much like buying gasoline from a pump labeled "premium" when every station has a different octane rating. [As consumers, we all know what that's like, particularly if we drive a car with a high compression engine and try to minimize gasoline cost]. As a result, uniform federal standards were eventually propogated. But it is worth noting that despite the present apparent sophistication of grain grades, current utilizers of grain may actually have less information about the quality of the commodity than did the fellow who was milling wheat in the last century.

This historical development emphasizes the need for a grading system, and the need that it be a universal, or common, language. And it also emphasizes a couple other important factors in a grading system. First, it must talk about things that are meaningful to the

people in the trade. Otherwise it isn't communicating anything that is worthwhile, and, rightfully, won't stand up. As the economy changes, grades may need to change in order to continue to be useful communication. Secondly, if there is a meaningful economic difference between differentiable lots of a commodity the trade usually finds some way of recognizing the difference. People who do not know the language are going to be penalized by their ignorance.

From the standpoint of the entire economy, a remarkable thing about a good universal grading system is that the words used to describe the commodity class mean pretty much the same to everybody in the trade. Hence, people can get on with their business without undue concern and without wasted time spent arguing the quality of the commodity which they are trading. A good grading system tends to make the whole economy more efficient. This forms the basis for an argument that grades ought to incorporate the biological contamination variables.

Perspective on Present Grades

Where do we now stand in the United States with respect to our grading system? Is it doing a good job? Is it doing the job we want it to do?

U.S. grades are, as you know, fixed by decree under provisions of the Grain Standards Act. The criteria are specified and remain fixed from year to year. Quantity available in each category varies as overall quality of the crop varies.

There are alternative grading systems. The Canadian grading system, for example, works somewhat different from ours. The Canada Grain Act provides for "statutory grades" of grain. These grades cover grain moving into terminal elevators. Each year the Committee on Western Grain Standards settles upon "standard export samples" for statutory grades and any other commercial grades needed to facilitate handling in that year. The standard export samples govern grades assigned to grain as it moves out of terminal elevators. The standard export samples must always exceed tolerances of standard samples within the statutory grade. Hence, the export grains must meet higher tolerances than the minimum official grade. The system works in practice to reduce variation within export grades. And, the exact grade standards may vary from year to year, depending on overall crop quality.

Let us now look at some evidence bearing on the issue of adequacy of the United States grading system:

1. There is surely evidence that our system is far from complete in the sense of assessing all relevant characteristics. For some groups of users it is necessary to perform special tests for variables which have value in their particular use. There are, for example, the widely-used tests for baking quality in wheat to attempt to measure the value to bakers of a particular lot of wheat. Feed processors must carefully evaluate the nutrient qualities of grain they buy. Oil content of soybeans must be evaluated by oil processors. Maltsters commonly test for uniform germination in barley. I am told they have developed special techniques to measure this variable which is important

for their use. I understand that the birdseed industry evaluates grain for color to make an attractive package--for people, not birds.

2. Another item of evidence in the overall completeness of the system is one that appears in the commodity pages of the newspaper every day. There are fairly significant price ranges within grades. This, to some degree at least, reflects the variation in utility within the grades for various users of the commodity. There is also addition of the non-grade protein variable in wheat, with the attendant rather wide price range for wheat within classes, depending on protein.

3. There is a whole set of so-called non-grade factors which are often added to further describe a lot of grain. For example: tough, smutty, ergoty, weevily, garlicky, and who knows what elseity. These appear to be factors which are important to users but yet which are not measured with a high degree of precision.

4. Another piece of evidence on grade adequacy is the one you have been confronted with the last day and a half. A whole set of biological contaminants of grains and animal by-products appear to be commercially important. The grading system apparently does not adequately cope with these factors which have an important bearing on the value of the commodity to the user. Consequently, there is little individual incentive to resolve the problem, because there are no penalties or rewards.

5. We tend to hear the rather vague kind of talk that the grading system is "not standing up,"--which I suppose means a variety of things. There is, I think, a feeling of uneasiness with some criteria of the present grading system and the grading standards.

6. Finally, in assessing the adequacy of the grading system, it is important to bear in mind the developments in production and handling grain. These relate to and have an important bearing on grain quality. Within the generation there has been a complete transition in the method of harvesting and handling wheat by the producer. We have shifted almost completely away from shocks, stacks, and a threshing machine to direct combining. This means the natural drying and conditioning which was allowed to take place in the shock and in the stack nowhere now enters the handling system.

We are just now in the midst of a similar change in corn harvesting. As you are aware, we are rapidly moving away from earcorn harvesting (where the corn dried naturally in the crib on the ear) to combining and field shelling. In the central cornbelt over half, and perhaps closer to three-fourths, of the corn is harvested as shelled corn. Three years ago only half the corn was harvested this way. Because of the practical impossibility of present corn hybreds drying on the stalk, corn must either enter the marketing system as a wet commodity, or it must be mechanically dried. Mechanical drying has brought with it the problem of stressed kernals, cracked kernals, over-drying, heat damage, and so on. At the other extreme, the new system has also brought with it under-drying or complete lack of drying. A whole host of quality variables are associated with high moisture grain

and with damaged grain. The grading system does not adequately reflect these variables. From the viewpoint of the farmer, it simply does not pay to undergo the cost of avoiding the damage.

In both wheat and corn there has consequently been introduced into the system a set of quality variables which always theoretically existed but did not in the past have a great deal of practical significance because of the method of harvesting and handling. At the same time, farm organization is changing such that relatively more feed-grain is sold off the farm. The commercial trade is thereby confronted with storage and handling problems which formerly were relegated to the farm where the grain was fed. Thus, simply the progress of technology must lead to questions of the adequacy of grain grading criteria.

Criteria for Grade System

Theoretically I suppose every commodity user has a somewhat different set of criteria that he is seeking in the commodity he buys. But the grading system probably cannot and should not incorporate all of these minute variables -- it simply would cost more than it would be worth. Nevertheless, if the grading system is going to work and be at its maximum effectiveness it ought to take into account the extent to which there are a common set of variables which are important to all users and to all members of the trade. A grading system must take into account the variables which are important to a broad segment of the trade and measure those which it is more efficient to do commonly than to do by every individual who has a potential interest in a particular lot of a commodity. This is the essence of the economic criteria of a good grading system.

To reiterate, it is not practically possible to measure all factors. This may be particularly true in regard to biological contaminants. However, if I understand the problem correctly, there appear to have developed a set of mutually-related variables which are significant in grain quality, which relate to biological contamination, and which have arisen largely since the present grain standards were enacted. These variables are the interrelated set of factors associated with time in storage, with moisture content, with damaged grain, with the grain-storage temperature, and with the storage facility in which the grain is located (i.e., the aeration equipment or the amount of air movement within the storage facility). These factors, in turn, become associated with some elements of biological contamination. It would seem that these variables ought to be quite readily amendable to measurement, ought to be important to all members of the trade, and ought to be measurable at relatively low cost. Consequently, they would seem to be factors that it would be economically efficient to incorporate into the grading system.

Grades Important in Export

A final point that is growing more important is that many of the grade factors become increasingly critical as we export more and more of our commodities. And we must remember that some of the common potential grading issues have not been terribly important among trade within the United States because of the "appeals" procedures for grain grading. If someone with an interest in the commodity doesn't particularly like the grade that it was given, it is a relatively simple matter (conceptually at least) to make

an appeal for the grade to be changed. However, as grain moves into export the appeal becomes a more cumbersome operation. It is one thing to appeal the grade given to a car still on track while you hold title to the grain, but it may be quite another to appeal the grade after the ship has been unloaded in Brussels or Bombay. It is, therefore, important that the grade properly assess arrival quality.

As we look at the export markets, it is also important to stress that our grading language say something that is meaningful to the prospective importers. Their requirements may, in fact, be different from ours. Their interpretation of specific grade factors may well be different. In short, our grading language simply may not communicate to prospective customers the set of variables in which they are interested. It ought to be pretty clear to all of us that every sprouted shipment, and every importer's port elevator full of high moisture corn can threaten our export market. Given the prospects that we are now facing with increasingly stiff competition in export markets, I think that we cannot be too careful of the terms of trade that we are communicating to prospective buyers. I fear we thought we could be a little careless of these considerations while we were living with the famine fantasy of the late 1950's and early 1960's. But as we now look at a grain surplus world it becomes increasingly clear that we are going to move our commodities into export only if we meet the competition of other exporters, and if we can demonstrate ability to fulfill the needs of prospective customers. We cannot afford to confuse customers with an unclear grade language, nor discourage them with an inadequate grade classification.

Conclusion

To recapitulate: I have taken a long way of expressing two basic points. The points are:

1. In the U. S. Market economy it is reasonable for individuals to resolve the quality issue of biological contamination if they are rewarded for supplying the desired qualities and/or penalized for the undesirable. By clearly and efficiently distinguishing the relevant variables in commodity quality, the grading system can help provide this incentive.

2. It is worth incorporating the biological contamination variables into grades if it is more efficient to measure them commonly for the market than individually for each user for whom they are important.

In closing, let me point out that there are alternatives to the reflection of the biological contamination variables in grades. One alternative is tight public regulation and policing of these quality variables. I think most of you would prefer to avoid that alternative.

THE GRANARY WEEVIL AS A CARRIER OF FUNGI AND PATHOGENIC BACTERIA

Phillip K. Harein
Professor and Extension Entomologist
Department of Entomology, Fisheries and Wildlife
University of Minnesota

In February 1967, the University of Minnesota sponsored a conference here in Minneapolis entitled "Grain and Cereal Products Sanitation". During this conference considerable effort was devoted to discussing contaminants in foods and feeds resulting from insects, microorganisms, birds and chemicals. However, time did not permit an indepth review of any one of these contaminants not to mention discussion on possible interrelationships among them.

It has been known for several years that the two major contaminants of grain and cereal products are insects and fungi. During these years an ever increasing amount of research was devoted to investigating the importance of stored-product insects. Simultaneously plant pathologists were accumulating information on storage fungi. However, studies on possible relationships between these two biological contaminants remained neglected until 1948 when cooperative work was initiated at the University of Minnesota between the Department of Entomology, Fisheries and Wildlife and the Department of Plant Pathology.

Results of this early work indicated a close association between the granary weevil, Sitophilus granarius (L) and various species of storage fungi. In general the data showed that the weevils introduced the microflora into their environment and provided optimum conditions for their development. In 1958 further research with granary weevils and fungi in columns of wheat indicated that localized infestations by the weevils initiated and promoted deterioration by storage fungi. The primary storage fungi associated with the weevil were species of Aspergillus and Penicillium.

Before proceeding further into this subject of biological interrelationships, it may be wise to review the development of the granary weevil. To my knowledge grain in Minnesota does not become infested with stored-grain insects, including the granary weevil, before harvest. However, any accumulation of post-harvest grain or cereal products is a target for this group of insects. The granary weevil is one of the four insect pests of stored-grain that usually has all of the immature stages developing within a kernel of grain. The adult weevil is somewhat cylindrical, ranges from light brown to almost black, and has a beak or snout on the front of the head. Its mouth is located on the end of this snout where it is an important tool for feeding or burrowing into its favorite food, whole kernels of grain. The female eats a small hole in a selected kernel of grain, deposits an egg in this cavity, and conceals it by sealing the hole with a gelatinous secretion. It is difficult to see this "cap" when she has completed the job. Within a few days a tiny white legless larva will hatch from the egg and begin to tunnel into the kernel. In about 2 months the insect will develop through the larval and pupal stages to become an adult. By this time a large percentage of the kernel has been consumed without having the insect break through to the outside environment. Shortly upon reaching the adult stage the weevil eats through to the outside of the kernel leaving a large and characteristic "emergency hole".

As I mentioned earlier, the association between the granary weevil and stored-grain fungi has been established. Little work has been reported, however, on the importance of the granary weevil in the introduction and dissemination of bacteria into stored grain and cereal products. In 1967 we diverted our efforts to this area. Laboratories for this project were provided at the University of Minnesota in the Department of Veterinary Microbiology and Public Health, the Department of Plant Pathology and the Department of Entomology, Fisheries, and Wildlife.

To begin this work we collected wheat infested with various stages of the granary weevil from two field infestations in Minnesota and from two laboratory colonies. The laboratory colonies were maintained at Kansas State University and the University of Minnesota. The emerged adult weevils were screened from the wheat within 24 hours after the sample was received and were washed immediately in various solutions to kill or remove bacteria on the surface of their bodies. The first washing was about 1-1/2 minutes in 100 milliliters of water containing 2% sodium hypochloride and 6 drops of Tween, $^{20\ (R)}$ an emulsifier. The second washing was 1/2 minute in 45% ethyl alcohol followed immediately by three washing of 1/2 minute each in sterile water. All surface disinfections hereafter were conducted as stated above. Effectiveness of the technique was determined periodically by dipping some of the treated insects in test tubes of nutrient broth. If the broth became turbid within 24 hours, the insect sample was considered contaminated and was discarded.

After surface disinfection of the weevils, two groups of 20 adults each were mascerated aseptically in 20 milliliters of saline solution with a mortar and pestle. Serial dilutions of the supernate were cultured at 37° in pour plates with three different agar preparations.

The laboratory colonies provided the source of immature weevils. Emerged adults and all kernels that were visibly cracked or broken were removed from the selected sample. The remaining kernels that contained weevil larvae or pupae were located with radiographs of the wheat. The radiographs were produced with a General Electric X-ray Inspection Unit using 20 kv and 5 ma with a 1 minute exposure on Type M Industrial film. The selected infested kernels were opened carefully and the immature weevels were transferred to sterile petri dishes when they were mascerated, diluted, and cultured as described for the adults.

The data revealed several points. For instance, no bacteria were found in the weevil larvaie, pupae, or pre-emergency adults that were removed aseptically from their host wheat kernels. The vacated, surface-disinfected kernels and the debris resulting from the developing weevil were also bacteriologically sterile. Subsequent culturing of adult weevils that emerged from the kernels in sterile petri dishes provided further evidence that the weevils were free of bacteria throughout the developmental stages.

However, the adult weevil became a carrier of many bacteria after emerging from the protective host kernel. Total bacterial counts per 20 adults from the Minnesota laboratory ranged from 67,000 to 410,000. The majority of the bacteria were members of the Klebsiella - Aerobacter group, plus Escherichia intermedia and Proteus rettgeri. A few were identified as species of Micrococcus.

It was evident that the Kansas State laboratory colony was cleaner than the Minnesota colony as shown by the relatively low bacterial counts of 4,000 to 5,000 per 20 adults and the bacterial species identified. Most were species of Micrococcus or the bacterium Bacillus subtilis. No coliforms were found in the Kansas State colony.

As expected, bacterial counts in the weevils from the field infestations were always greater than in weevils from the laboratory colonies. The range was 555,000 to 4,600,00 per 20 adults. The majority of the bacteria from the field insects were Bacillus spp., Micrococcus spp., Streptococcus spp., P. vulgaris and other coliforms. Serratia marcescens was also isolated from one of the samples.

A brief review of the bacterial species isolated helps to understand the importance of this granary weevil - bacteria association. Micrococcus spp., E. intermedia, and B. subtilis are commonly found in soil, dust, water, and various food products. However, some species of Proteus have been associated with various human pathological conditions. The pathogenicity of this bacterium is related with human eye and ear infections, pleuritis, peritonitis, and supperative abscesses. Proteus has also been found responsible for certain infections of the digestive tracts of humans especially in infants. The Klebsiella - Aerobacter group is pathogenic or potentially pathogenic to warm blooded animals, including man. The Streptococci are responsible for many human diseases, perhaps a greater variety than any other taxonomic group of bacteria. In addition, they have a marked tendency to occur in mixed and secondary infections with other pathogenic bacteria. S. marcescens is found in water, soil, food, and in other insects. Bacteria in the genus Serratia are generally free-living saprophytes but occasionally are found associated with, and possibly casual relation to, pathological processes in man.

In conclusion, the granary weevil is a carrier of several bacterial species including those that are pathogenic to man. It should be considered a potential source and disseminator of bacteria into grain and cereal products.

MICRO-ORGANISMS ASSOCIATED WITH THE

LESSER MEALWORM

Ernesto De las Casas, Research Fellow
Department of Entomology
University of Minnesota

I shall begin by describing the lesser mealworm and some of its habits before discussing any association with microorganisms. The adult is a shiny, black, robust insect, about 7 mm long with the head narrower than the thorax. It has short legs and well developed wings but is a slow flier. The lesser mealworm is nocturnal in habit; it does not like light and enjoys and prefers warm, damp places. Its average life span is about 4 to 5 months although we have kept them alive in the laboratory for over a year.

A full grown larva measures between 12 to 15 mm in length. It is dark in color, very active and it has strong mandibles which enable it to feed on hard materials.

Literature published on the lesser mealworm has dealt primarily with feeding observations, incidence in poultry broiler houses and control. The lesser mealworm is known to be widely distributed. It has been collected from many different types of habitats; in flour mill basements where it feeds on damp or musty flour or grain, in bakeries hidden in wooden cracks, in nests of birds and bats, and even in rotten vegetables. In general, it has been considered a secondary infester of grain and related cereal products.

Larvae and adults feed and develop successfully in a mixture of warm litter and poultry droppings. Frequently, this species is present in very large numbers, together with several other species of insects and mites. Chickens search through the litter looking for the lesser mealworm. The birds may ingest diseased organisms or parasites present in this insect that otherwise may not be easily available.

In a recent survey the beetles were found in all the 150 broiler houses inspected in Northern Georgia and all the 98 broiler houses examined in Arkansas. Sometimes the beetles were present in extremely high numbers. In our observations around Minnesota we have found the lesser mealworm in 95% of the brooder houses examined.

In recent years, there has been increased interest in the lesser mealworm. Some of the reasons are:

a). It has been considered one of the most common carriers of caecal worms and other poultry parasites.

b). Investigators demonstrated that the lesser mealworm was involved in the transmission of the leukosis virus or "Marek's" disease in chickens. The interest increased when they were able to demonstrate that the virus was carried by the beetle not only externally but also internally.

c). Until 1958, this insect was assumed to be strictly phytophagous or saprophagous. However, there are several reports in which it has been observed burrowing into and feeding on the flesh and internal organs of dead and dying chickens.

d). Effective control under field conditions has not been practiced. The habits of the lesser mealworm account for some of the difficulties encountered in their control. They burrow into the litter, crawl into cracks in the foundation and tunnel through the walls into the insulation. This makes it difficult to remove them or to get lethal doses of insecticides to the infested area.

Good management and sanitation are the main concerns in efficient poultry operations. Beetle infestations are not advisable. Managers become concerned when they observe the infestation of the lesser mealworm and they realize that the insect can be a possible vector of diseases.

As far as we know there are no published results on the relationship between the lesser mealworm and bacteria. About two years ago we initiated a cooperative program between the Department of Microbiology and Public Health and the Department of Entomology, Fisheries, and Wildlife at the University of Minnesota.

Our initial steps were laboratory experiments where we explored the capabilities of the lesser mealworm to ingest and to survive with bacteria such as Salmonella typhimurium. We recovered S. typhimurium in different amounts from the feed (dog food) and from within the insects after several storage periods. The number of colonies of bacteria per gram of food decreased gradually for about 2 weeks. After this time it remained relatively constant (58 million the 1st week to less than 10 million after 4 weeks). We learned that under our laboratory conditions the lesser mealworms ingested bacteria in amounts that varied from less than a million up to 30 million colonies per insect. They survived with them, excreted them and suffered no apparent ill effects. We also killed and stored some of the infected adults in a sterile environment for 45 days. We recovered S. typhimurium from each of the adults tested with counts ranging from 50 to 1/2 million colonies per individual insect.

The capability of the lesser mealworm, alive or dead, to carry Salmonellae was evident. Our next step was to screen adults collected under natural conditions for the presence of these and other microorganisms.

We collected the lesser mealworm from different poultry brooder houses and we counted and identified aerobic bacteria within 400 individual adults. Our results indicated that the lesser mealworm is able to harbor, internally, considerable numbers of bacterial contaminates. Counts ranged from 8,000 to 10,000,000 colonies per individual insect. The highest counts were 24 hours after the field collection. Starvation tended to decrease the bacterial count in the insects. No bacteria were found in some following an eight-day starvation period and the number of bacteria-free insects increased as starvation time progressed. It is likely that the amount of food inside their intestines is one of the factors involved.

However, the capability of the lesser mealworm to harbor a considerable amount of contaminants after dying from starvation was evident. One insect still contained 750,000 bacteria.

The gram positive bacteria present in the previous counts included Micrococcus spp. and Streptococcus spp. in more than 50% of the adults and Corynebacterium spp. and Baccilus spp. in about 20%. These bacteria are also abundant in the intestine of healthy men and animals, soil, decomposing organic matter, poultry excrement and air. Staphylococcus aureus was isolated from one infestation site. It is of major interest because of its pathogenic significance to man and animals. This bacteria is known to be involved in cases of arthritis, dermatitis and eye diseases in poultry. We are familiar with "Staph" infections in humans and we know it is a widely recognized problem of increasing importance.

Among the gram negative bacteria, species of the Escherichia group were present in more than 50% of the adults. These bacteria, are commonly found in soil, organic matter, etc., which accounts for their abundance in the beetles. Some of the other gram negative bacteria isolated included Serratia marcescens, Proteus spp. and Pseudomonas aeuriginosa. Although found in a low percentage of the insects, their internal presence should not be overlooked since these bacteria are considered as pathogens or potential pathogens to warm blooded animals including man. They are usually associated with secondary infections or as secondary invaders.

A significant finding from this preliminary screening was the isolation of Salmonella saint paul and a few pathogenic serotypes of Escherichia coli.

This finding led us to the next step in our project in which only Salmonella species and pathogenic serotypes of E. coli were our main concern. We increased our field collections and we analyzed 1,000 individual adult lesser mealworms for the internal presence of these two bacteria.

Table #1 shows the five species of Salmonella that we recovered internally from the adults and the number of insects that were positive for each bacterial species. A total of 2.2% of the adults were internal carriers of Salmonellae. The same species of Salmonella were recovered several times from the excrement of these beetles. All these species are a problem in poultry especially in poults, and we know they are all potential disease producers for other animals, including man. Although the incidence of Salmonellae was only 2.2% in the insects, the five different species isolated indicated that the lesser mealworm can harbor and reflect those microorganisms that are present in the environment of the poultry houses.

Table #2 shows the number and some of the pathogenic E. coli serotypes recovered from the adults. Twenty-six different serotypes were isolated from 15.1% of the insects. It is known that serotypes such as 01a and 02a are the cause of severe intestinal and respiratory problems in young birds. About 10.8% of the insects were internal carriers of pathogenic E. coli for poultry.

Serotypes of E. coli pathogenic to other animals including man were present in 4.3% of the adult lesser mealworm. Serotypes such as 0127 and 0124, isolated from less than 1% of the insects, have been implicated in outbreaks of diarrhea in humans, mainly infants.

In summary, we believe that the relatively constant migration of the lesser mealworm in search of optimum feeding or breeding areas ultimately results in their exposure to most of the environment within poultry houses. As they concentrate in selected areas their metabolic activity contributes to the temperature and moisture within the litter. This creates favorable sites for the survival and reproduction of microorganisms. The lesser meal worm serves as an indicator for microorganisms in the poultry environment and can be considered as a potential source and effective disseminator of pathogenic bacteria for man and animals.

Table No. 1

Species of Salmonella recovered from 1,000 surface disinfected adults collected from field infestations.

Salmonella spp.	Adults Positive
typhimurium var. copenhagen	4
S. saint paul	3
S. chester	11
S. worthington	3
S. heidelberg	1

Table No. 2

Some of the pathogenic serotypes of E. coli recovered from 1,000 surface disinfected adult lesser mealworms.

Pathogenic to poultry	Adults positive
01a	1.4%
022	0.7%
0112 ab	1.0%
088	1.0%
Total 17	10.8%

Pathogenic to other animals including humans, calves, pigs, and swine.

08	2.0%
0124	0.9%
0127	0.8%
Total 9	4.3%

COMPUTER AND THE USE OF MULTIVARIATE STATISTICS IN STORED GRAIN RESEARCH[1]

R. N. Sinha
Research Station, Canada Department of Agriculture
Winnipeg 19, Manitoba

Contamination of grain and stored products by several biological and non-biological agents, although generally accepted as fact, is difficult to document. The data available are fragmentary, have been gathered by various methods, vary in reliability, and are scattered in various journals of different disciplines. Even when masses of data and suitable statistical techniques have been available the calculations involved were so formidable that few attempted the complicated correlations between bio-contamination and environment. This has changed with the computer revolution which began in the 1950's, and in 1969 computer technology is applied in all areas of daily life where volume and complexity of data are beyond human capabilities.

In the high-speed digital computer research workers studying bio-contamination in grain and foodstuffs now have a powerful tool, it permits data reduction, statistical analysis, problem simulation, and theory development. Thus it is essential that the grain researcher be familiar with the potential of this new technology, its attributes and its limitations. He must also be informed of pertinent statistical procedures and have suitable programs available.

Today's digital computer is a high-speed automated version of the desk calculator which has substituted punched cards for keys and transistors for mechanical wheels. The computer has 5 basic units:

1. Input through which instructions and data are supplied
2. Control which regulates the operation of the component parts of the computer system
3. Memory which stores information as electric charges on magnetized elements
4. Arithmetic unit which computes at a fantastic speed
5. Output which provides the results of computation in a comprehensible form

Ideally a grain researcher who uses multivariate analyses should collaborate with a statistician, and have assistance from a mathematician, a programmer, and a systems engineer. If one is resourceful, such cooperation can be achieved. For example, at present the computer center at the University of Manitoba makes available to the University staff over 200 IBM scientific subroutines and many prepackaged programs. To use these instant programs the investigator must know to what question he wishes to have answers; the statistician then determines the specific statistical procedure to be used and the programmer selects, or if necessary writes an appropriate program.

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[1]Contribution No.364 from Canada Agriculture Research Station, Winnipeg

The biologist should be familiar with the organisms under investigation and their environment before he attempts to use any multivariate procedure. Of course, the methods of collection and assessment of samples must not be changed in the middle of a long-term study. Prior to the analysis the investigator should study the distribution patterns for each variable and when necessary transform the data to obtain normality in distribution.

Effective use of the computer and multivariate techniques demands that one, with or without the aid of a statistician, be able to recognize the method necessary to solve the specific problem. It is not essential, however, to understand all mathematical details of the method and the operation of the computer (the domain of the systems engineer) before a problem can be solved.

Five useful multivariate statistical methods are briefly discussed below:

A. Multiple linear regression analysis This procedure is best known and examines the relationship between one dependent variable (criterion) and 2 or more independent variables (predictors). One can predict future events or analyze cause and effect relations among past events. For example, the fat acidity of stored grain at some future time can be predicted by analysing data collected in the past and determining the relationship between levels of fat acidity in the grain samples and infestation by several species of fungi.

B. Multiple-discriminant analysis This popular procedure is used to examine or predict the group to which particular individuals belong on the basis of a set of attributes; the attributes are measured as continuous variables. The aim of this procedure is to assign a member or a sample to one population or the other and not to determine what is the best way of dividing heterogeneous material into populations or groups since these are predetermined. For example, one may use this method to assign on the basis of infestation by various fungi, several grain samples into either toxic or nontoxic groups.

C. Principal component analysis This method helps explain in simplified terms, unclear relations among several variables when each has been measured in randomly selected samples from a population. Hence, it is most useful in exploratory studies as it aims to provide a parsimonious and meaningful summary of a large body of data while exploring the nature of relationships among the important variables. Because the method works towards a theoretical model from a set of measurements (Kendall 1965) it is useful in generating hypotheses.

   For example, we conducted a principal-component analysis on 32 variables, each measured in a total of 8135 samples collected at monthly intervals from two 500 bushel wheat bulks stored in Winnipeg from 1959 to 1967 (Sinha et al. 1969b). The variables were classified as environmental, microbiological, and entomological. Eleven principal components extracted from the 32 x 32 correlation matrix accounted for 59% of the total variability. The general principal component, time, affected all major field storage fungi and moisture content of the grain; insects and mites were not affected. Temperature and moisture both affected abundance of mites, insects, and fungi;

temperature is a more important variable than moisture for most mites and insects, whereas moisture is the more important variable for most fungi. Interrelations between fungi and arthropods in a bulk grain ecosystem were confirmed statistically.

D. Factor analysis This method analyzes the measurements taken by established procedures on a set of variables from each of many samples. It examines the intercorrelations to determine whether the variation represented can be explained by several basic categories smaller than the total number of variables. Unlike the principle component analysis, factor analysis starts with a hypothetical model which is tested to determine whether it conforms with the experimental data. If it does the parameters are estimated (Kendall 1965). Both analyses have 3 objectives: parsimony, orthogonality, and meaningfulness. The terms 'factor analysis' and 'factor analytic procedures' are often used as generic terms for several multivariate techniques aimed at analyzing intercorrelations within a set of variables (Cooley and Lohnes 1962).

E. Canonical correlation analysis The foregoing multivariate methods have assumed univariate analysis on one side of the cause and effect statement and multivariate analysis on the other, even though the simple one to one or one to many cause and effect relationship may be rare in nature. Canonical correlation analysis, developed by Hotelling (1935), assesses the maximum correlation between the linear functions of a set of dependent (criterion) and a set of independent (predictor) variables, instead of using one dependent variable at a time.

For example, Sinha et al. (1969a) used three canonical correlation analyses to determine relationships among groups of variables in two eight-year-old bulk grain ecosystems:

I. Seed germination and field fungis vs environment.
II. Storage fungi vs environment.
III. Seed germination and field fungi vs storage fungi.

The analyses revealed that in aging wheat bulks invasion of the storage fungi Chaetomium, Aspergillus, Rhizopus and the actinomycete Streptomyces, is significantly related to both loss in grain viability and decrease in the field fungi Alternaria, Helminthosporium, and Gonatobotrys. The non-biological environment factors, temperature and the physical conditions of storage, are extremely important in reduction of field fungi whereas storage fungi such as Penicillium and Aspergillus are affected mostly by temperature, moisture content of the grain, and time.

Today's society is computer-oriented. Consequently, I urge the worker in grain research to define his questions accurately and to seek quantitative answers. This will not only open exciting new horizons, but will enable him to utilize the powerful problem-solving techniques that have yielded handsome dividends for his colleagues in the physical sciences.

LITERATURE CITED AND SELECTED REFERENCES

Anderson, T. W. 1958. Introduction to multivariate statistical analysis. John Wiley and Sons, Inc., New York

Borko, H. 1962. Applications of high speed digital computers in the behavioral sciences. Prentice Hall Inc., Englewood Cliffs, N. J. 1962.

Cooley, W. W. and Lohnes, P. R. 1962. Multivariate procedures for the behavioral sciences. John Wiley and Sons Inc., New York.

Harmann, H. H. 1967. Modern factor analysis. 2nd Ed. University of Chicago Press, Chicago.

Hotelling, H. 1936. Relations between two sets of variates. Biometrika 28: 321-377.

Kendall, M. G. 1965. A course in multivariate analyses. Charles Griffin and Co., Ltd., London.

Morrison, D. F. 1964. Multivariate statistical method. McGraw Hill Book Co., New York.

Seal, H. 1964. Multivariate statistical analysis for biologists. Methuen and Co., Ltd., London.

Sinha, R. N., H. A. H. Wallace, and F. Chebib. 1969. Canonical correlations of seed viability, seed-borne fungi, and environment in bulk grain ecosystems. Canadian J. Bot. 47: 27-34 pp.

Sinha, R. N., H. A. H. Wallace, and F. Chebib. 1969b. Principal component analysis of interrelations among fungi, mites, and insects in grain bulk ecosystems. Ecology (In press).

Snedecor, G. W. and W. G. Cochran. 1967. Statistical Methods. The Iowa State University Press, Ames.

Wilks, S. S. 1964. Mathematical statistics. John Wiley and Sons Inc., New York. 44 pp.

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