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Fungi in Relation to Health in Man and Animal.

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I.—INTRODUCTION.

A.—General.

The fungi harmful to man and animal may be divided into three main groups according to the nature of their effects :—

(*a*) Those growing on the skin and mucous membranes and in the organs, for example, those causing ringworm and aspergillosis.

(*b*) Those, which grow "wild" and contain toxic substances within themselves, for example, poisonous mushrooms (toadstools).

(*c*) Poisonous symbiotic and parasitic fungi attacking foodstuffs :

(1) Those containing the poison within themselves (ergot).

(2) Those producing harmful substances in the foodstuffs on which they grow.

The fungi concerned in groups (*a*) and (*b*) have been the subject of many investigations and are extensively referred to in the literature. The information obtained through these investigations is definite and conclusive, whilst our knowledge of the fungi concerned in group (*c*), and the results obtained by experiments with these fungi, are inconclusive, contradictory and by no means definite. It is for these reasons that it is proposed to deal only with the last group of fungi.

The following literature may be consulted for information with regard to the first two groups of fungi :—

Group (*a*).—Pammel (1911), Fröhner (1919), Fröhner (1922), Seiffert (1920), Byam and Archibald (1921), Hutyra and Marek (1922), Romanov (1928), Bull (1929), Schäfer (1929), Whitfield (1929), Woolridge (1929), Morris (1931), Steyn (1931), Datta (1932), Seren (1932), and Thomson and Fabian (1932).

Seren (1932) gives an excellent historical review of fungi causing disease through growing in animal and human tissues ; also his experiments are of great interest.

Group (*b*).—Bruinsma (1906), Kobert (1906), Pammel (1911), Heffter (1924, Vol. 2, No. 2), Silberbauer and Mirvish (1927), Petri (1930), and Glaister (1931).

B.—Historical.

Group A (c).—Fungus-infected Foodstuffs.

Husemann, Hilger and Husemann (1882) refer to cases of poisoning in Italy caused by bread prepared from fungus-infected maize, and state that an extract prepared from this bread was poisonous and contained an alkaloidal substance.

These authors refer to a bitter, loliin, which is contained in the seed of *Lolium temulentum* Linn. (darnel, drabok), but make no mention of fungi being concerned in the causation of the toxicity of darnel.

Kobert (1906) states that in poisonous darnel there is a fungus between the hyaline layer and the aleuron cells and that this fungus does not affect the viability of the plant. Antze claimed to have isolated two alkaloids, loliin and temulentin, and temulentic acid from darnel. Hofmeister found a crystalline base, temulin ($C_7H_{12}N_2O$), which was absent in fungus-free darnel, to be the active principle. Temulin has narcotic and mydriatic effects, and the lethal dose for cats is 0·25 gm. per Kg. bodyweight. According to Hofmeister fungus-infected darnel does not contain more than 0·06 per cent. of the alkaloid temulin. In France it is held that darnel is most poisonous before it ripens.

Quoting Woronin, Kobert continues that in Russia fungus-infected rye was responsible for darnel-like poisoning in human beings. The rye grains were infected with *Fusareum roseum* Link, "*Gibberella saubinitii*" (ascophoric form of the former), "*Helminthosporum herbarum*," "*Epicoccum neglectum*," "*Trichothecium roseum*," "*Eurotium herbariorum*" (ascophoric form of "*Aspergillus glaucus*"), "*Hymenula glumarum*," "*Cladochytrium graminis*", etc.

Similar symptoms in human beings and in animals were caused by rye in France in 1890. All these rye grains were infected with the same fungus, which Prillieux and Delacroix termed "*Endoconidium temulentum*" (in the conidia form).

Kobert also refers to the toxic nature of the so-called sleepy grass ("*Stipa vaseyi*"), and suggests that its toxicity is due to a parasitic fungus. Marsh and Clawson (1929) have, however, proved this plant toxic to horses and sheep and make no mention of the presence of fungi on the plant.

Pammel (1911) refers at length to fungi. Certain fungi (for example, "*Mucor Rouxii*" in China) are used in the preparation of yeast and alcoholic drinks as they change starch into sugar, which in turn liberates alcohol on further fermentation. In experiments conducted by Gamgee in 1868 two cows were fed a mixture of corn meal, hay and corn smut (*Ustilago zeae* Beck). The ration of the one animal was moistened whilst the other received the mixture in a dry state. In the course of three weeks these two animals consumed forty-two pounds of corn smut. The cow receiving the dry ration lost in condition despite a voracious appetite, whilst the other animal remained in good health and gained in weight. Further cases are quoted by Pammel in which corn smut was fed to heifers without any ill-effects. Corn smut fed in large amounts also to cows for a period of forty-nine days produced no harmful effects. The milk yield was normal and none of the experimental animals aborted. In addition to these experiments numbers of others, in which cattle have ingested large amounts of corn smut, are referred to.

Ustilago avenae (Pers.) Jens. (oat smut) is said to produce a sore throat when present in large quantities. The sore throat is caused by irritation and is not due to this fungus growing in the mucous membrane. Barley smut [*Ustilago nuda* (Jes) Kell and Sw.], wheat smut [*Ustilago tritici* (Pers.) Jens.] and *Ustilago panici-glauci* (Wallr.) resemble oat smut in its harmful effects on man and animal. From the last-named fungus Power isolated a small amount of ergotin and some farmers consider that this fungus causes abortion.

Tilletia foetens (B and C) Trel. stinking smut or bunt) and *Tilletia Tritici* (Bjerk) Wint (wheat bunt) are referred to as causing a bad colour and dark colour in flour, but nothing is mentioned about their toxic properties.

The rust of oats, wheat and grass (*Puccinia spp.*) are liable to cause severe irritation of the buccal and nasal mucous membranes.

Referring to *Penicillium glaucum* Link. (blue mould) Pammel (1911) says that it is widely distributed on decaying fruit and certainly is not pathogenic apart from the fact that it may sometimes cause mycotic stomatitis. *Aspergillus glaucus* (L.) Link is stated not to be pathogenic but probably causes the development of a poisonous substance in the substratum. "Staggers" or "mad staggers" (enzootic cerebritis), is said to be caused by feeding maize, which is attacked by this fungus. Reference is also made to the possibility of its causing mycotic stomatitis.

Pammel (1911) and Long (1917) refer to *Claviceps purpurea* (Fr.) Tul. Pammel states that *Diplodia zeae* (Schw.) Lèv. occurs in the ears of mealies and may be responsible for forage poisoning. Foodstuffs attacked by *Fusarium roseum* Link are supposed to be poisonous. Pammel also refers to the toxicity of darnel being due to a fungus.

Brown (Editorial, 1916) found a "yeast" (Torula) and two moulds, namely "*Mucor erectus*" and "*Aspergillus oryzae*" in the root of *Mesembryanthemum Mahoni* N.E. Br., from which the natives of South Africa prepare a fermenting agent, which is used in the manufacture of a drink named "Khadi" and as a rising principle for bread.

Fröhner (1919) states that "*Claviceps purpurea*" (ergot) usually occurs on rye but may also be found on wheat, oats, barley and grasses. As active principles he describes conutin (or secacornutin), which causes contraction of the uterus; sphazelinic acid, which produces necrosis due to hyaline degeneration and thrombosis of the peripheral arterioles; and ergotic acid, which is a narcotic and has no action on the uterus. The symptoms of ergot poisoning are described as follows: gastro-intestinal irritation; necrotic stomatitis; gangrene and mummification of extreme parts of the body; uterus contractions; and nervous disturbances. This fungus, which in itself is poisonous, has been responsible for many cases of poisoning in man and animal. Fröhner describes botanic and spectroscopic methods for the detection of ergot in grain and hay.

Fröhner (1919) attributes the following symptoms to foodstuffs attacked by "*Mucor mucedo*," "*Mucor racemosus*," "*Mucor stolonifer*," "*Mucor phycomyces*," "*Aspergillus glaucus*," "*Penicillium glaucum*" and "*Oidium lactis*": inappetence, colic, tympanites, constipation and diarrhoea. In some cases salivation, difficult deglutition, vomiting, icterus, polyuria, nephritis, cystitis, dizziness, staggering, pronounced depression, paralysis of the extremities, hindquarters, tongue, urinary bladder, ears and retina, and general paralysis were present. Pushing, bellowing, shivering, convulsions, epilepti-form spasms, profuse perspiration, imperceptible pulse, reddish-brown discolouration of the conjunctiva and rapid loss in condition were also seen.

The post-mortem lesions described are : gastro-enteritis with haemorrhages and erosions ; accumulation of serum in the brain cavities and in the arachnoidal sac ; hyperaemia and oedema of the brain and spinal cord ; haemorrhagic fluid in the peritoneal cavity ; cystitis, nephritis ; peritonitis and acute yellow atrophy of the liver. In some cases the autopsy is negative.

"*Tilletia Caries*" (on wheat), "*Ustilago carbo*" (on wheat, oats, barley and pasture grasses), "*Ustilago moidis*" (on mealies), "*Ustilago longissima*" (on grasses) and "*Ustilago echinata*" (on *Phalaris* and *Phragmites spp.*) are held responsible for the following symptoms in animals (Fröhner, 1919) : paralysis of pharynx, oesophagus and tongue ; weakness ; swaying gait ; staggering ; complete paralysis of the motor and sensory nerves ; constipation ; diarrhoea, swelling of the eyelids ; lachrymation ; and catarrh of the upper air-passages ; and pregnant animals may abort.

The post-mortem in many cases reveals no lesions. Sometimes irritation of the gastro-intestinal tract and the air passages are found.

The following symptoms are ascribed by Fröhner (1919) to foodstuffs infected with *Puccinia graminis, Puccinia straminis, Puccinia coronata, Puccinia arundinacea*, and *Uromyces apiculatus :* inflammation of the skin, lips, cheeks, eyelids, and head ; urticaria ; severe itching ; conjunctivitis and inflammation of the buccal mucous membrane, pharynx and gastro-intestinal tract.

The post-mortem reveals, apart from the skin lesions, haemorrhagic gastro-enteritis, nephritis and cystitis, and haemorrhages in the serous membranes.

Furthermore, Fröhner mentions that the following fungi are poisonous : "*Polydesmus exitiosus*" (on rape), "*Polythrincium trifolii*" (on clover) and "*Epichloë typhina,*" (on grasses). The symptoms produced by foodstuffs attacked by these fungi are similar to those described for *Ustilago* and *Puccinia spp.*

The treatment consists in the administration of purgatives, calomel being preferred on account of its disinfecting properties ; furthermore, creatin, and, as chemical antidotes, tannin, tannoform and iodine. For the rest the treatment is symptomatic.

Fröhner also refers to the fact that foodstuffs infected with these fungi can at times be taken in large amounts with impunity.

Fröhner considers the suggestions that darnel is toxic only when infected with a fungus as acceptable.

Furthermore, Fröhner (1919, p. 391) states that (a) "*Poa aquatica*" attacked by "*Ustilago longissima,*" and (b) "*Phragmites communis*" infected with "*Puccinia arundinacea*" are poisonous. "*Peronospora viticola*" causes colic, tympanites, constipation, diarrhoea and abortion in cows. "*Peronospora Herniariae*" and "*Peronospora Viciae*" are also stated to be poisonous.

Mitchell [1918 (a)] produced hyperaesthesia, inco-ordination of movements, muscular tremors, dyspnoea and an accelerated and weak pulse in cattle by feeding them on the fungus "*Claviceps paspali,*" which is of frequent occurrence on "*Paspalum dilatatum*" pastures. He was unable to produce abortion in pregnant animals and mummification as are found in "*Claviceps purpurea*" poisoning. A meal of nine pounds of *paspalum* heads, which were badly attacked by the fungus, sufficed to produce well-defined symptoms, whilst 8 oz. of pure sclerotia caused quite recognizable symptoms. The symptoms appeared two days after feeding and recovery was very slow.

Mitchell (1918) proved that maize infected with "*Diplodia zeae*" causes inco-ordination of movement and paralysis in cattle. He was able to produce symptoms of poisoning within two days after having fed 20 lb. of artificially infected maize to bovines.

Mitchell (1918) also fed 19½ lb. of maize artificially infected with "*Mucor mucedo*" to an ox without the animal suffering any ill-effects.

Fröhner (1922) adds the group of yeast fungi (*Hefepilze*) to the four groups described by him in his text book on toxicology (Fröhner, 1919). These yeast fungi are responsible for alcohol fermentation. He ascribes the following symptoms of poisoning in animals to foodstuffs, in which this group of fungi have caused fermentation: cerebral stimulation with subsequent narcosis and paralysis.

Hutyra and Marek (1922) (Vol. 3, p. 187) state that according to observations "*Ustilago Maidis*" causes gout in birds.

Thomson and Sifton (1922) discuss poisoning by ergot and fungus-infected foodstuffs and refer to the fact that foodstuffs infected with certain moulds have been found poisonous whilst on other occasions they proved innocuous when fed in large mounts, although the same fungi were present. A specific case is mentioned in which a strain of "*Aspergillus fumigatus*" from Italy was very poisonous whilst the same strain obtained from Germany was very slightly or not at all toxic.

The experiments of Graham, Bruechner and Pontius (Thomson and Sifton, 1922) with mouldy hay have thrown much light on some mysterious cases of poisoning with fungus-infected foodstuffs. They isolated an anaerobic botulinus-like organism from mouldy hay, which had caused typical cases of forage poisoning. The moulds present in the hay apparently provide the necessary anaerobic conditions for the growth and propagation of this botulinus-like bacillus. Graham and his collaborators furthermore produced typical forage poisoning by feeding pure cultures of this bacillus to animals and also succeeded in producing immunisation to this organism. The symptoms of poisoning described closely resemble those described by Theiler and his collaborators (1927) in parabotulism (Lamsiekte) on South Africa.

Danckwortt (1926) sounds a warning note with regard to the use of solvents in the extraction of oil from foodstuffs, for example, the use of ether and trichlorethylene. Ether is easily decomposed by air and light and poisonous substances may be the result as has been proved to be the case. Trichlorethylene may exert poisonous effects as, owing to its enormous surface area on the foodstuffs, it is absorbed very rapidly. Poisoning with such extracted foodstuffs may erroneously be attributed to fungi which may be present on these foodstuffs.

Jarmai (1925) produced visceral and articular gout in geese by force-feeding them with maize infected with "*Penicillium glaucum*." Negative results were, however, obtained when an artificial culture of this fungus was fed to or injected intravenously into fowls. Furthermore, cases of uraemia in ducks caused by mouldy maize and in pigeons caused by mouldy wheat are mentioned.

Lander (1926) refers to the toxicity of ergot and darnel, but makes no mention of the toxicity of the latter being due to a fungus.

Heyne (1927) mentions that *Mucor dubius* Wehmer, *Mucor javanicus* Wehmer and *Rhizopus oryzae* Wert et Prinsen Geerlings are used in China and Java in the preparation of yeast *ragi*. The last-named fungus renders the less digestible ingredients of legume seeds more assimilable.

Theiler (1927) describes deplodiosis (*Diplodia zeae* Schwz. Lev) in cattle and sheep. Feeding experiments with pigs and horses were negative. Three pounds of mealies infected with "*Diplodia zeae*" ingested in three days caused poisoning in sheep. Two pounds of maize artificially infected with this fungus caused poisoning in sheep within two days. The symptoms disappear within a few days after the discontinuation of the feeding with "*Diplodia zeae*" infected maize.

No symptoms of poisoning could be produced by feeding the mycelium alone.

The symptoms described by Theiler closely resemble those reported by Mitchell (1918). The post-mortem revealed catarrhal enteritis and hyperaemia of the lungs and kidneys.

Elsässer (1928) describes vomiting and a foetic diarrhoea in pigs and, in those with an unpigmented skin, an intensely itching vesicular eczema after feeding on mouldy barley that had been imported from America.

Lührs (1928) also refers to this fungus-infected American barley, which caused vomiting in pigs. Lührs attempted feeding this barley to pigs but they refused to take it. A pig was then drenched with 1 pound of this barley and vomiting occurred within fifteen minutes after drenching.

Lührs considered biogenic amines, for example, cholin, responsible for the toxic effects of this barley, and described a method of differentiating between normal barley and the mouldy American barley. He was able to detoxicate this barley by boiling it with a 2 per cent. sodium carbonate and then neutralising with 10 per cent. hydrochloric acid and again boiling for thirty minutes.

Stang [1928 and 1928 (a)] states that horses, cattle, sheep and laboratory animals took the mouldy American barley which caused vomiting in pigs, with impunity.

Stang [1928 (a)] examined the barley microscopically and found hyphae and spores of "*Fusarium roseum*" whose form of development occurred as "*Gibberella Saubinettii*."

Danckwortt (1929) found up to 0·095 per cent. ammonia in the harmful American barley whilst normal barley contained 0·073 per cent. He, therefore, confirmed Altenberg's findings that there is no appreciable increase in the ammonia or volatile amine bases in mouldy rye. Danckwortt also found no difference of any significance in the sulphuretted hydrogen content of the American barley and normal barley.

The following is quoted from an abstract (Editorial 1929) dealing with the abovementioned mouldy American barley: "On the other hand, the investigation of similar samples by the Director of the German Institute of Milling failed to reveal any fungus or toxamine, but showed the presence of undetermined bacteria which fermented barley dough with a copious production of gas and caused a repulsive butyric acid-like smell. It is believed that these bacteria cause fermentation and the decomposition of proteids in the animal stomach, the products of which are toxic to pigs, animals which are known to be highly susceptible to this kind of poisoning."

Miessner and Schoop (1929) detected spores of "*Fusarium roseum*" in the mouldy American barley to which reference has already been made and succeeded in growing this fungus on artificial media. The following fungi were found in specimens of this barley: a red pigment forming yeast, "*Cladosporium herbarum*," "*Alternaria spp.*," and "*Fusarium roseum*."

These fungi proved to be non-pathogenic to guinea pigs and mice. The three first named fungi had no detrimental effect on pigs to whose ration large amounts of pure cultures of these fungi were added; also when parenterally administered they produced no ill-effects. "*Fusarium roseum*" cultures added to the foods of pigs caused these animals to refuse the food altogether. Pigs dosed by means of a stomach tube with large amounts of "*Fusarium roseum*" cultures showed increased defaecation and inappetence for twenty-four hours.

Miessner and Schoop regarded "*Fusarium roseum*" as the causative factor in the production of poisonous substances in this mouldy American barley.

Oppermann and Doenecke (1929) also refer to this harmful American barley.

The following is a passage from a publication by Dickson and his co-publishers (1930): "The water extract from barley severely infected by scab ("*Gibberella Saubinettii*") finely ground and extracted for four to six hours, was found to produce acute vomiting in pigs, and became more active when freed from protein, polysaccharides and nitrogenous substances precipitable with tannic acid. The active substances or materials appear to be associated with the fractions containing glucoside or basic nitrogen compounds."

Heller, Caskey and Penquite (1930) during their investigations into the toxicity of smuts have suffered ill-effects due to the inhalation of the smut spores. These ill-effects were sensitivity of the upper pulmonary tract, increased heart action, headache and feeling of nausea. These authors investigated the effects of grain-sorghum smuts on rats, guinea pigs, rabbits, fowls, horses and cows. Rats, guinea pigs, rabbits and fowls were fed on a well-balanced diet to which these smut spores had been added. In addition sorghum plants of which fully 70 per cent. had smut-infected heads, were fed to horses, milk cows and young cattle. All the above animals were fed over prolonged periods without any ill-effects as to their condition, growth, milk yield, egg yield and reproduction.

Müller (1930) attributed the following symptoms in a horse to poisoning with hay infected with *Aspergillus* (definite species not mentioned): colic, inappetance, pushing against stable wall, pronounced perspiration, muscular tremors, extreme excitement, pronounced salivation, dyspnoea, fever, cyanosis, frequent urination, charging with head against stable wall, turning somersaults, staggering, falling, exhaustion, and an imperceptible pulse. Death occurred twenty-four hours after the onset of symptoms.

Autopsy revealed gastro-enteritis: meningoencephalitis acute haemorrhagica; incipient acute swelling of the spleen: and turbid swelling of the liver, kidneys and myocard. Müller found the hay, part of which this animal had consumed, infected with "*Aspergillus*" and considered this the cause of death.

Mundkur and Cochran (1930) write: "During the autumn of 1928, hogs and poultry in Iowa were extensively poisoned by the consumption of barley contaminated by *Gibberella Saubinettii* the perithecia of which were present on the surface of 4-8 per cent. of the grain. Feeding experiments were carried out on hogs, chickens and guinea pigs, all of which rejected an exclusive barley diet, while the two first-named developed symptoms of nausea and lost weight."

Roche, Rokstedt and Dickson (1930) state that cattle, sheep and poultry on Wisconsin farms may be fed on barley infected with "*Gibberella Saubinettii*" (scab) without suffering any ill-effects, whereas pigs, dogs, horses and man are susceptible to low percentages of badly scabbed kernels. They suggest that scab infected fodder be fed to ruminants and poultry and in this way prevent wastage.

Barger (1931) published an excellent monograph in which he discusses the historical, botanical, chemical, pharmacological and clinical, and the pharmaceutical and forensic aspects, of "Ergot and Ergotism." See article "Poisoning of Human Beings by Weeds contained in Cereals (Bread poisoning)" published elsewhere in this Report (Darnel poisoning).

Bürgi (1931) states that "*Mucor*," "*Penicillium*" and "*Aspergillus*" are to be considered with regard to poisoning caused by fungus-infected foodstuffs and also refers to the fact that animals may at times ingest such foodstuffs over prolonged periods without any apparent detrimental effects.

Bürgi has seen several cases of colic in horses as a result of eating fungoid hay and also gastro-enteritis and other symptoms of poisoning, most with exitus letalis, in horses which had taken mouldy bread.

Bürgi, furthermore, states that with regard to fermented hay the following organisms (according to Düggeli) are concerned: "*Mucor*," "*Aspergillus*," "*Bacterium coli*," "*Oidium lactis*," *Bacterium fluorescens*," "*Bacillus mesentericus*" and "*Actinomyces thermophyles liquefaciens*." Most of these organisms are destroyed at a temperature of 75°C. The oxidation process, however, continues on pure chemical lines through the catalytic action of iron and manganese combinations, with the result that the temperature in fermenting hay stacks may reach 280°C. From this moist mass water, carbon dioxide, saltpetre combinations, karomel, furfurol, acetic and formic acid can be distilled. Furfurol on aldehyde in cheap alcoholic drinks has been proved poisonous to dogs and fowls.

Bürgi also refers to poisoning with fresh hay, the symptoms of which are inappetence, drowsiness, fever, accelerated pulse, colic, diarrhoea, tympanites, heart weakness and abdominal pulsation. Death occurs with symptoms of dyspnoea and heart failure. On autopsy pronounced hyperaemia of the small intestine and oedema of the lungs were present.

It was thought that cumarin is the cause of the trouble but Fröhner found that cumarin and other scents of fresh hay cause more or less severe intestinal irritation, but no actual poisoning. Poisoning with fresh hay is, therefore, not clearly understood.

Mains, Vestal and Curtin (1931) fed "*Gibberella Saubinettii*" infected barley to pigs with the result that these animals took scarcely enough to maintain life. Amounts of this barley, not exceeding 10 per cent. of the total ration, were however, safely given to pigs. This barley, which contained 58 per cent. of scab, was successfully fed to cattle as 50 per cent. of the grain ration, and to fowls as 20 per cent. of the ration.

Richner (1931) states that mustiness and mouldiness in cereal seed grain are liable to occur when the moisture content of the grain exceeds 15 per cent. The principle fungi concerned were "*Penicillium*," "*Citromyces*," and "*Aspergillus*," followed by "*Mucor*" spp., "*Rhizopus nigricans*," "*Fusarium culmorum*," "*Fusarium herbarum*," "*Alternaria tenuis*," "*Dematium pullulans*," "*Trichothecium roseum*" and "*Actinomycetes*."

Shofield (Roderick and Schalk, 1931) produced a very acute anaemia in rabbits with a destruction of 50 per cent. of the red blood cells by feeding these animals on aqueous extracts of mouldy sweet clover. The clotting time of the blood was delayed. Unfortunately no reference is made to the fungi concerned.

Csukas (1932) describes cases of acute inflammation of the upper air-passages in horses and in some of the human beings attending these horses. In each case he found the hay or straw infected with fungi, but he was unable to produce this affection experimentally with the suspected fungus infected hay or straw. No mention is made of the kinds of fungi or organisms concerned, except in one case in which streptococci, staphylococci, actinomyces, streptrotrix and a thick rod-like bacterium were isolated from the bedding straw.

Riedl (1932) describes laminitis in horses due to the feeding of fermented sugar beet.

(C.) The Toxic Constituents of Fungi and Fungus-infected Foodstuffs.

The toxic constituents of fungi and fungus-infected foodstuffs can according to the information at our disposal, be divided into (*a*) those that are produced and contained in the fungus itself (*Claviceps purpurea* and *Claviceps paspali*) and (*b*) those that are produced by fungi growing on foodstuffs and causing certain chemical changes in the constituents of such foodstuffs.

(a) *Toxic constituents of* Claviceps purpurea *and* Claviceps paspali.

Barber (1931) and all text-books on pharmacology and toxicology deal extensively with ergot (*Claviceps purpurea*) and ergotoxine, ergotinine, tyramin and histamin are generally accepted as its active constituents (Milks, 1930). Mitchell (1918), who proved *Claviceps paspali* toxic to cattle, makes no reference to its active principles.

Heffter (1923) mentions that Dale and Ewins detected acetylcholin in ergot. Acetylcholin is much more poinonous than cholin, but is very unstable. The symptoms of poisoning are similar to those causes by cholin. Recently it was found that the action of ergot as an ecbolic is due in the first place to an " unknown constituent " and not to ergotoxine and ergotamin (Editorial, 1932).

Gieger (1932) separated an amorphous alkaloid from the oil of *Claviceps paspali*. Less than 0·1 gm. of this alkaloid proved fatal to a guinea pig.

(b) *The toxic constituents of fungus-infected foodstuffs.*

This aspect of mouldy foodstuffs has been repeatedly investigated with varying and inconclusive results.

Bodin and Gautier (Pammel, 1911, p. 265) state that "*Aspergillus fumigatus*" produces a bacteria-like toxin when grown on media containing a mixture of protein and carbohydrate. This toxin exerts its chief actions on the nervous system causing muscular convulsions. Rabbits and dogs are much more susceptible to this toxin than guinea pigs, cats, mice and white rats.

Fröhner (1919) states that poisoning with fungi is caused not by their spores entering the blood circulation in a physical way but by toxins formed by the fungi. The toxin of smuts has an irritant action on mucous membranes and a paralytic effect on the centre of deglutition and the spinal cord. Very little is known of the production and nature of fungus toxins, probably these are products of decomposition of constituents of the substances on which the fungi grow. Leber found irritant toxins, resembling the phlogosin of streptococci and staphylococci, in cultures of "*Aspergillus fumigatus*" and "*Penicillium glaucum*," whilst Buss detected a poisonous substance in "*Oidium albicans*" (Fröhner, 1919).

Seiffert (1920) mentions that Ceni and Besta, and Bodin extracted specific toxins from the mycelia of "*Aspergillus fumigatus*."

Czapek (1921) states that the formation of oxalic acid by fungi growing on sugar containing media is a well-known phenomenon. "*Peziza sclerotiorum*" (*Sclerotinia Libertiana* Fuck), forms more oxalic acid from sugar when the substratum contains calcium than when it is calcium-free. It is stated that "*Aspergillus niger*" forms oxalic acid not only when growing on substrata containing sugar but also on those containing salts of organic acids, albumoses, amino acids, and to a lesser extent on those containing glycerine and vegetable fats. The origin of the nitrogen is of importance in the formation of oxalic acid by fungi on sugar substrata in as much as no oxalic acid is formed when ammonium chloride or ammonium sulphate is added, whilst it is readily liberated when peptone serves as origin of the nitrogen. It is furthermore mentioned that "*Saccharomyces Hansenii*" forms oxalic acid from sugars and carbohydrates.

Many investigators consider that the toxicity of fungus-infected foodstuffs is due to the fungi secreting toxins on the feed (Thomson and Sifton, 1922).

Danckwortt (1926) accepts that fungi themselves are non-poisonous and that they cause the formation of harmful substances in the substrata. These substances probably are products of decomposition of proteins contained in the foodstuffs attacked by fungi. Biogenic amines, some of which are poisonous, may be formed. Abderhalden proved amino acids harmless to dogs.

Of the biogenic amines, which are likely to be formed in fungus-infected foodstuffs and which may be responsible for the poisoning caused by such foodstuffs, cholin, which is a product of decomposition of phosphatides, has received most attention.

Cholin is a constituent of most plant cells and is also present in many animal tissues to the extent of 0·01 to 0·03 per cent. Cholin is not considered very poisonous as it is rapidly oxidised in the animal tissues. Frogs are killed by 0·1 to 0·25 gm. cholin chloride administered subcutaneously. There is cessation of respiration and death with paralysis. 0·5 to 1·0 gm. per Kg. body-weight administered to mammals causes salivation, lachrymation, increased peristalsis, weakness of extremities and death with symptons of respiratory paralysis. When administered intravenously spasms occur before death. Cats are more susceptible than rabbits (Heffter, 1923).

Sollman (1922, p. 325) gives the lethal intravenous dose of Cholin for mammals as 1–2 mg. per Kg. body-weight.

Cholin is a parasympathetic stimulant, like muscarin, with many of its actions similar to those of pilocarpine and physostigmine.

According to Trier (1931) putrefactive organisms decompose cholin into the ten times more poisonous neurin.*

Neither Brieger nor Ruckert nor Vogt succeeded in isolating neurin from cultures of "*Oidium lactis*," "*Cholrera virrions*," "*Penicillium*" or "*Algae*," which were allowed to decompose through bacterial action (Heffter, 1923).

* Führer (1932) found 0·09–0·1 mg. acetylcholin per gram bodyweight lethal to mice and 0·2–0·23 mg. per gram bodyweight lethal to frogs (*Rana temporaria*).

Schroeter and Strassburger (1931) extracted from mouldy American barley a poisonous substance, which on subcutaneous administration to frogs and mice caused spasms and paralysis, which in most cases ended fatally within ten to eighteen hours. Control extracts from healthy barley proved to be non-toxic.

Schroeter and Strassburger found free cholin in the above barley. They fed cholin-butyric-acid-ester to pigs with negative results. On injecting subcutaneously into 30–35 Kg. pigs 0·4 gm. cholin-butyric-acid-ester chlorhydrate in 10 c.c. distilled water the following symptoms were developed: immediate restlessness, squealing, climbing the walls of the sty, extremely accelerated pulse within one minute after injection, pronounced dyspnoea and collapse. Improvement set in within thirty minutes after injection, complete recovery resulting within eight hours.

From the results of their investigations Schroeter and Strassburger concluded that cholin or some easily hydrolysable cholin-butyric-acid-esters were the cause of the harmfulness of the mouldy American barley.

Starek [Schroeter and Strassburger (1931)] suggests that the fungi or bacteria found on the American barley decompose lecithin into cholin or easily hydrolysable esters of cholin.

According to Boehm (Schroeter and Strassburger, 1931) cotton-seed cakes contain toxic amounts of free cholin.

Theiler (1927) reported negative results with regard to feeding experiments on sheep with the mycelium of "*Diplodia zeae*." Van der Byl found aldehyde, alcohol and acids in the cultures of this fungus. Theiler suggests that under certain conditions large amounts of alcohol and aldehyde may be formed by this fungus and may then prove harmful. The intestinal catarrh caused by "*Diplodia zeae*" infected mealies was, according to Theiler, due probably to the acids present in the cultures.

Lührs (1928) refers to the fact that healthy barley contains 0·035 per cent. cholin and states that, according to his investigations, there was a marked increase in the cholin content of the harmful mouldy American barley due to the action of the fungi. He continues that owing to the fact that pigs are very susceptible to these biogenic amines, it is quite acceptable that cholin is the toxic ingredient of this barley as only pigs were affected.

Danckwortt (1929) examined the already referred to mouldy American barley chemically. He excluded poisoning with heavy metals, alkaloids and prussic acid. He found no appreciable difference in the ammonia and hydrogen sulphide content of this barley and normal barley. Unfortunately it is at present still impossible to determine by chemical means qualitatively and quantitatively the labile biogenic amines.

According to Wernery (1929) trimethylamin was detected in a specimen of bran which had caused harmful effects in cows and which contained many micro-organisms. Altenburg Wernery, (1929) isolated tyramin from the substrata of fungi cultures.

Trimethylamin, which is a typical product of decomposition of all proteids, causes increased reflexes, accelerated respiration and pronounced convulsions, which are followed by deep coma.

Tyramin, which is a very common product of protein putrefaction, has an adrenaline-like action and also causes contraction of the uterus.

Dickson and his co-publishers (1930) found the active substances of the above harmful mouldy American barley to be associated with those fractions of the extracts containing glucoside or basic nitrogen compounds.

According to Bürgi (1931) Valentin detected in fungi cultures alkaloidal substances, which had an irritant and lethal action on experimental animals. Bürgi also mentions that Di Pietro isolated from the spores of *Penicillium toxicum* a glucoside, which caused spastic-paretic symptoms with an increase in reflexes and muscular tone. Ceni and Besti (Bürgi, 1931) isolated from "*Aspergillus fumigatus*" and "*Aspergillus flavescens*" a spasm-producing toxin and a paralytic toxin from "*Aspergillus niger*" and "*Penicillium*" spp.

With regard to poisoning with fresh hay it was thought that cumarin, the characteristic sweet smelling constituent of fresh hay, was responsible for the harmful effects, but Fröhner found cumarin and other scents of fresh hay not to have any detrimental effects on the system apart from causing a more or less severe intestinal irritation.

Roderick and Schalk (1931) administered cumarin per os in large amounts over prolonged periods to rabbits with no apparent ill-effects. They were unable to extract the toxic principle of damaged sweet clover.

Seren (1932) quotes the views of Valentin, Leber, di Pietro, Ceni and Berta, and Bodin and Gautier, with regard to the toxic constituents of mouldy foodstuffs. These views have already been referred to.

D.—Immunity to Poisoning with Fungus-infected Foodstuffs.

(a) *Natural Immunity.*

In plant poisoning acquired immunity and acquired tolerance must be clearly distinguished from each other. The term acquired immunity should be used only in connection with plants which contain toxalbumins as active principles, which cause the development of antibodies in the body, whilst acquired tolerance should be spoken of in connection with those plants, which when repeatedly taken by animals, cause the development of a resistance other than that brought about by the production of antibodies. As the nature of the active principles produced in foodstuffs by the growth of fungi is unknown only the term immunity will be used in this article.

Mitchell (1918) writes: "Horses, mules and donkeys would appear not to be susceptible, as these animals graze over the same area where cattle are dying from the disease without suffering any harmful results. No cases have been recorded of goats or pigs having been affected."

Thomson and Sifton (1922) state that horses and mules are the animals chiefly affected, cattle, sheep, pigs and poultry being apparently very resistant, if not immune to the effects of fungi.

Theiler (1927) recorded negative results in feeding experiments with "*Diplodia zeae*" infected maize on horses and pigs, whilst cattle and sheep developed well-marked symptoms and even died.

Stang (1928) states that horses, cattle, sheep and laboratory animals took the mouldy American barley in all forms, whereas pigs refused it and when forced to take it the latter animals developed inappetence and some even vomited.

Roche, Boksteds and Dickson (1930) referring to scab infected barley write: "It has been found that cattle, sheep, and poultry on Wisconsin farm may be fed on barley infected with scab (*Gibberella Saubinettii*) without adverse effects, whereas pigs, horses and dogs, as well as man, cannot tolerate even low percentages of badly scabbed kernels. It is suggested that a considerable saving may be effected by the utilization of infected fodder for the ruminants and poultry, since it must otherwise be sold at a heavy discount."

Mains, Nestal and Curtis (1931) state that barley containing up to 58 per cent. of scab ("*Gibberella Saubinettii*") was successfully fed to cattle as 50 per cent. of the grain feed ration and to fowls as 20 per cent. of the ration, whilst pigs hardly took enough of this barley to maintain life.

(b) *Acquired Immunity.*

Mitchell (1918) expressed the view that an ox which developed no symptoms of poisoning although it had ingested a larger quantity of Diplodia-infected maize than the two bovines which developed symptoms, may have developed a tolerance resulting from an attack in the preceding year.

Seiffert (1920) failed to produce an immunity in guinea pigs to "*Aspergillus fumigatus*" by injecting them subcutaneously and intravenously with virulent material and also subcutaneously with nucleoproteid solutions prepared from "*Aspergillus fumigatus*" and subsequently testing their immunity by injecting virulent spore emulsions intravenously.

II.—ONDERSTEPOORT EXPERIMENTS.

Specimens of fungus-infected bran, mealies, hay and ensilage, which were suspected of having caused harmful effects in human beings (maize) and stock (bran, mealies, hay and ensilage), have repeatedly been received at Onderstepoort for investigation.

Maize showing a 100 per cent. infection of fungi was fed to fowls (60 grams) and to rabbits in quantities up to 847 gm. without any deleterious effects. No additional food was given during the period of feeding.

In the following table is contained a summary of the experiments conducted by the author in the course of the last four years at Onderstepoort with fungus-infected foodstuffs.

EXPERIMENTS WITH FUNGUS-INFECTED FOODSTUFFS CONDUCTED AT ONDERSTEPOORT.

Animal.	Method of administration.	Material fed or drenched.	Period of feeding or drenching.	Total amount fed or drenched.	Result.
Rabbit A.....	Fed (no additional food given)	100 + mouldy bran infected with *Rhizopus* (probably *R. nigricans*) a *Fusarium* sp. and bacteria	16 days.....	1070 grams..	Negative.
Rabbit B.....	,, ,,	*Fusarium moniliforme* culture on maize. (No. 550—see Addendum by Dr. Doidge.)	7 days.....	680 grams..	,,
Rabbit C.....	,, ,,	,, ,, ,,	7 days.....	1000 grams..	,,
Rabbit D.....	Per stomach tube	*Fusarium moniliforme* culture on Raulin's medium. The " mould skin " was separated from the fluid, washed several times in tap water and given to this animal (No. 550)	3 days.....	60 grams..	,,
Rabbit E.....	,, ,,	,, ,, ,,	,,	,,	,,
Rabbit F.....	,, ,,	The fluid, from which the " mould skin " drenched to rabbits D and E had been removed, was given to this rabbit	3 days.....	240 c.c.....	,,
Rabbit G.....	,, ,,	,, ,, ,,	,, ...	,, ...	,,
Rabbit H....	,, ,,	Pure Raulin's medium (control).....	2 days.....	240 c.c.....	,,
Rabbit I.....	,, ,,	,, ,, ...	,, ...	,, ...	,,
Rabbit J.....	Fed (no additional food given)	*Fusarium moniliforme* var. *sub-glutinans* culture on maize (No. 602)	7 days.....	900 grams..	,,
Rabbit K.....	Per stomach tube	*Fusarium moniliforme* var. *sub-glutinans* culture on Raulin's medium. The mould skin was separated from the fluid, washed several times in tapwater and then given to this animal	3 days.....	60 grams..	On the fourth day of the experiment this animal was found partially paralysed; death occurred the same day. *Post-mortem appearances:* Pronounced hyperaemia of lungs, dilation of both heart ventricles.

EXPERIMENTS WITH FUNGUS-INFECTED FOODSTUFFS CONDUCTED AT ONDERSTEPOORT—(*continued*).

Animal.	Method of administration.	Material fed or drenched.	Period of feeding or drenching.	Total amount fed or drenched.	Result.
Rabbit L.....	Per stomach tube	*Fusarium moniliforme* var *sub-glutinans* culture on Raulin's medium. The mould skin was separated from the fluid, washed several times in tapwater and then given to this animal	3 days.....	60 grams..	On the eleventh day of the experiment this animal was partially paralysed, the paralysis progressed until animal was prostrate and unable to move. It died on the third day after onset of symptoms. *Post-mortem appearances.* Pronounced hyperaemia of lungs and liver, dilatation of both heart ventricles—urinary bladder markedly distended with ordinary urine.
Rabbit M.....	Per stomach tube	The fluid, from which the " mould skin " drenched to rabbits K and L had been removed, was given to this rabbit	3 days.....	240 c.c.....	Negative.
Rabbit N.....	,, ,,	,, ,, ,,	,, ...	,, ...	,,
Rabbit O.....	Fed (no additional food given)	*Fusarium graminearum* Schabe culture on maize (No. 622)	7 days.....	600 grams..	,,
Rabbit P.....	,, ,,	,, ,, ,,	,, ...	310 grams..	,,
Rabbit Q.....	Per stomach tube	*Fusarium graminearum* Schwabe culture on Raulin's medium. The " mould skin " was separated from the fluid, washed several times in tap water and then given to this animal (No. 622)	3 days.....	60 grams..	,,
Rabbit R.....	,, ,,	,, ,, ,,	,, ...	,, ...	,,

EXPERIMENTS WITH FUNGUS-INFECTED FOODSTUFFS CONDUCTED AT ONDERSTEPOORT—(*continued*).

Animal.	Method of administration.	Material fed or drenched.	Period of feeding or drenching.	Total amount fed or drenched.	Result.
Rabbit S.....	Per stomach tube	The fluid, from which the " mould skin " drenched to rabbits Q and R had been removed, was drenched to this animal	3 days.....	240 c.c.....	Negative.
Rabbit T.....	,, ,,	,, ,, ,,	,, ...	,, ...	,,
Rabbit U.....	,, ,,	*Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium. The " mould skin " was separated from the fluid, washed repeatedly in tap water and drenched to this rabbit (No. 631)	4 days.....	80 grams..	,,
Rabbit V.....	,, ,,	,, ,, ,,	,, ...	,, ..	,,
Rabbit W....	,, ,,	The fluid, from which the " mould skin " drenched to rabbits U and V had been removed, was given to this rabbit	5 days.....	300 c.c.....	,,
Rabbit X.....	,, ,,	,, ,, ,,	,, ...	,, ...	,,
Rabbit Y.....	,, ,,	*Fusarium moniliforme* var. *subglutinans* cultures on Raulin's medium. The " mould skin " was separated from the fluid, washed repeatedly in tap water and drenched to this rabbit (No. 632)	5 days.....	100 grams..	,,
Rabbit Z.....	,, ,,	,, ,, ,,	,, ...	,, ..	,,
Rabbit Aa....	,, ,,	The fluid, from which the " mould skin " drenched to rabbits Y and Z had been removed, was given to this animal	5 days.....	300 c.c.....	,,
Rabbit Ab....	,, ,,	,, ,, ,,	,, ...	,, ..	,,
Rabbit Ac....	,, ,,	*Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium. The " mould skin " was treated as above and given to this animal (No. 665)	5 days.....	100 grams..	,,
Rabbit Ad....	,, ,,	,, ,, ,,	,, ...	,, ..	,,
Rabbit Ae....	,, ,,	The fluid, from which the " mould skin " drenched to rabbits Ac and Ad had been removed, was given to this animal	5 days.....	300 c.c.....	,,
Rabbit Af....	,, ,,	,, ,, ,,	,, ...	,, ...	,,

EXPERIMENTS WITH FUNGUS-INFECTED FOODSTUFFS CONDUCTED AT ONDERSTEPOORT—(*continued*).

Animal.	Method of administration.	Material fed or drenched.	Period of feeding or drenching.	Total amount fed or drenched.	Result.
Rabbit Ag....	Per stomach tube	*Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium. The " mould skin," treated as above, was given to this animal (No. 284)	5 days.....	100 grams..	Negative.
Rabbit Ah....	,, ,,	,, ,, ,,	,, ...	,, ..	,,
Rabbit Ai....	,, ,,	The fluid, from which the " mould skin " drenched to rabbits Ag and Ah had been removed, was given to this rabbit	5 days.....	300 c.c.....	,,
Rabbit Aj....	,, ,,	,, ,, ,,	,, ...	,, ...	,,
Rabbit Ak....	,, ,,	*Fusarium graminearum* Schwabe culture on Raulin's medium. The " mould skin," treated as above, was given to this rabbit (No. 622)	2 days.....	120 grams..	,,
Rabbit Al....	,, ,,	The fluid, from which the " mould skin " drenched to rabbit Ak had been removed, was given to this rabbit	2 days.....	200 c.c.....	,,
Rabbit Am...	,, ,,	*Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium. The " mould skin," treated as above was given to this rabbit (No. 222)	2 days.....	120 grams..	,,
Rabbit An....	,, ,,	The fluid, from which the " mould skin " drenched to rabbit Am had been removed, was given to this rabbit	2 days.....	200 c.c.....	,,
Rabbit Ao....	,, ,,	*Fusarium moniliforme* var. *subglutinans* Wr. and Rkg. culture on Raulin's medium. The " mould skin," treated as above, was given to this animal (No. 223)	,, ...	120 grams..	,,
Rabbit Ap....	,, ,,	The fluid, from which the " mould skin " drenched to rabbit Ao had been removed, was given to this rabbit	,, ...	200 c.c.....	,,
Rabbit Aq....	,, ,,	*Fusarium moniliforme* var. *subglutinans* Wr. and Rkg. culture on Raulin's medium. The " mould skin," treated as above, was given to this animal (No. 224)	,, ...	120 grams..	,,

EXPERIMENTS WITH FUNGUS-INFECTED FOODSTUFFS CONDUCTED AT ONDERSTEPOORT—(*continued*).

Animal.	Method of administration.	Material fed or drenched.	Period of feeding or drenching.	Total amount fed or drenched.	Result.
Rabbit Ar....	Per stomach tube	The fluid, from which the " mould skin " drenched to rabbit Aq had been removed, was given to this rabbit	2 days.....	200 c.c.....	Negative.
Rabbit As....	,, ,,	*Fusarium moniliforme* Sh. culture on Raulin's medium. The " mould skin," treated as above, was given to this rabbit (No. 220)	,, ...	120 grams..	,,
Rabbit At....	,, ,,	The fluid, from which the "mould skin" drenched to rabbit As had been removed, was given to this rabbit	,, ...	200 c.c.....	,,
Rabbit Au....	,, ,,	*Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium. The " mould skin," treated as above, was given to this rabbit (No. 602)	1 day.....	60 grams..	,,
Rabbit Av....	,, ,,	The fluid, from which the "mould skin" drenched to rabbit Au had been removed, was given to this rabbit	1 day.....	80 c.c.....	,,
Rabbit Aw ..	,, ,,	8 day old *Fusarium moniliforme* var. *subglutinans* culture on mealie meal (No. 602)	2 days.....	100 grams..	,,
Rabbit Ax....	,, ,,	16 day old *Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium. The " mould skin," treated as above, was given to this rabbit (No. 602)	1 day.....	50 grams..	,,
Rabbit Ay....	,, ,,	The fluid, from which the " mould skin " drenched to rabbit Ax had been removed, was given to this rabbit	1 day.....	80 c.c.....	,,
Rabbit Az....	Fed (no additional food given)	Mealie meal infected with *Fusarium moniliforme* var. *subglutinans* (No. 602)	13 days.....	1230 grams..	,,
Rabbit Ba....	,, ,,	16 day old *Fusarium moniliforme* var. *subglutinans* culture on mealie meal (No. 602)	2 days.....	110 grams..	,,
Rabbit Bb....	,, ,,	20 day old *Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium. The " mould skin," treated as above, was given to this rabbit (No. 602)	1 day.....	60 grams..	,,
Rabbit Bc....	,, ,,	The fluid, from which the " mould skin " drenched to rabbit Bb had been removed was given to this rabbit	1 day.....	120 c.c.....	,,

EXPERIMENTS WITH FUNGUS-INFECTED FOODSTUFFS CONDUCTED AT ONDERSTEPOORT—*(continued)*.

Animal.	Method of administration.	Material fed or drenched.	Period of feeding or drenching.	Total amount fed or drenched.	Result.
Rabbit Bd....	Fed (no additional food given)	20 day old *Fusarium moniliforme* var. *subglutinans* culture on maize (No. 602)	2 days.....	100 grams..	Negative.
Rabbit Be....	,, ,,	25 day old *Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium. The "mould skin," treated as above, was given to this rabbit (No. 602)	1 day.....	60 grams..	,,
Rabbit Bf....	,, ,,	The fluid, from which the "mould skin" drenched to rabbit Be had been removed, was given to this rabbit (No. 602)	,, ...	120 c.c.....	,,
Rabbit Bg....	,, ,,	25 day old *Fusarium moniliforme* var. *subglutinans* culture on maize, kept at temperature of 36°C. for last five days (No. 602)	2 days.....	100 grams..	,,
Rabbit Bh....	,, ,,	25 day old *Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium, kept at room temperature. The "mould skin," treated as above, was given to this rabbit (No. 602)	1 day.....	60 grams..	,,
Rabbit Bi....	,, ,,	The fluid, from which the "mould skin" drenched to rabbit Bh had been removed, was given to this rabbit (No. 602)	,, ...	120 c.c.....	,,
Rabbit Bj....	,, ,,	25 day old *Fusarium moniliforme* var. *subglutinans* culture on maize kept at room temperature (No. 602)	2 days.....	100 grams..	,,
Rabbit Bk....	,, ,,	25 day old *Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium kept at a temperature of 2°C. for last six days. The "mould skin," treated as above, was given to this rabbit (No. 602)	1 day.....	60 grams..	,,
Rabbit Bl....	,, ,,	The fluid, from which the "mould skin" drenched to rabbit Bk had been removed, was given to this rabbit (No. 602)	,, ...	120 c.c.....	,,
Rabbit Bm...	,, ,,	25 day old *Fusarium moniliforme* var. *subglutinans* culture on maize kept at a temperature of 2°C. (No. 602)	,, ...	50 grams..	,,

EXPERIMENTS WITH FUNGUS-INFECTED FOODSTUFFS CONDUCTED AT ONDERSTEPOORT—(*continued*).

Animal.	Method of administration.	Material fed or drenched.	Period of feeding or drenching.	Total amount fed or drenched.	Result.
Rabbit Bn....	Fed (no additional food given)	30 day old *Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium. The " mould skin," treated as above, was given to this rabbit	1 day.....	60 grams..	Negative.
Rabbit Bo....	,, ,,	The fluid, from which the " mould skin " drenched to rabbit Bn had been removed, was given to this rabbit (No. 602)	,, ...	120 c.c.....	,,
Rabbit Bp....	,, ,,	30 day old *Fusarium moniliforme* var. *subglutinans* culture on maize (No. 602)	,, ...	50 grams..	,,
Rabbit Bq....	,, ,,	35 day old *Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium. The " mould skin," treated as above, was given to this rabbit (No. 602)	,, ...	60 grams..	,,
Rabbit Br....	,, ,,	The fluid, from which the " mould skin " drenched to rabbit Bq had been removed, was given to this rabbit (No. 602)	,, ...	120 c.c.....	,,
Rabbit Bs....	,, ,,	35 day old *Fusarium moniliforme* var. *subglutinans* culture on maize (No. 602)	,, ...	50 grams..	,,
Rabbit Bt....	,, ,,	40 day old *Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium. The " mould skin," treated as above, was given to this rabbit (No. 602)	,, ...	60 grams..	,,
Rabbit Bu....	,, ,,	The fluid, from which the " mould skin " drenched to rabbit Bt had been removed, was given to this rabbit (No. 602)	,, ...	120 c.c.....	,,
Rabbit Bv....	,, ,,	40 day old *Fusarium moniliforme* var. *subglutinans* culture on maize (No. 602)	,, ...	50 grams..	,,
Rabbit Bw...	,, ,,	45 day old *Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium. The " mould skin," treated as above, was given to this rabbit (No. 602)	,, ...	60 grams..	,,
Rabbit Bx....	,, ,,	The fluid, from which the " mould skin " drenched to rabbit Bw had been removed, was given to this rabbit (No. 602)	,, ...	120 c.c.....	,,

EXPERIMENTS WITH FUNGUS-INFECTED FOODSTUFFS CONDUCTED AT ONDERSTEPOORT—(*continued*).

Animal.	Method of administration.	Material fed or drenched.	Period of feeding or drenching.	Total amount fed or drenched.	Result.
Rabbit By....	Fed (no additional food given)	45 day old *Fusarium moniliforme* var. *subglutinans* culture on maize (No. 602)	1 day.....	50 grams..	Negative.
Rabbit Bz....	,, ,,	Fungus-infected *Lolium temulentum* Linn. (See Leemann's article on darnel in this Journal.)	38 days.....	3370 grams..	,,
Rabbit Ca....	,, ,,	,, ,, ,,	,, ...	3225 grams..	,,
Pig 789 (7 months old)	A small quantity of milk added	Fungus-infected *Lolium temulentum* Linn. (See Leemann's article on darnel in this Journal.)	3 days.....	4000 grams..	,,
Dog (number lost)	,, ,,	,, ,, ,,	10 days.....	1250 grams..	,,
Pig 791 (8 months old)	,, ,, starved for 18 hours before being fed	45 day old culture of *Fusarium moniliforme* Sh. (var. *subglutinans* Wr. and Rkg.) (No. 222) (on maize)	24 hours.....	2000 grams..	,,
Pig 792 (8 months old)	,, ,,	45 day old culture of *Fusarium moniliforme* Sh. var. *subglutinans* Wr. and Rkg. on barley (No. 222)..	,, ...	2400 grams..	,,
Pig 790 (8 months old)	,, ,,	48 day old culture of *Fusarium graminearum* Schwabe on (barley grain) maize (No. 630)	2 hours....	400 grams..	,,
Pig 789 (8 months old)	,, ,,	42 day old culture of *Fusarium graminearum* Schwabe on barley grain (No. 630)	24 hours...	2400 grams..	,,

From the foregoing table it is evident that, (*a*) a rabbit ingested 1070 gm. of bran very badly infected with a *Rhizopus* sp. (probably "*Rhizopus nigricans*)," a *Fusarium* sp., and bacteria, in the course of sixteen days without suffering any ill-effects; (*b*) rabbits received cultures of "*Fusarium moniliforme*" on maize and on Raulin's medium without any detrimental effects; (*c*) fifty rabbits received cultures of "*Fusarium moniliforme var. subglutinans*" on Raulin's medium, two of these rabbits, which received the washed "mould skin," died with symptoms of paralysis, whilst the others developed no symptoms of poisoning. Twelve rabbits and one pig received cultures of this fungus grown on maize without suffering any ill-effects; the cultures were grown for varying periods and at different temperatures. One pig ingested 2400 grams of a 45 day old culture of this fungus on barley grain without suffering any harmful effects; (*d*) four rabbits received cultures of *Fusarium graminearum* Schwabe on Raulin's medium without developing any symptoms of poisoning; a rabbit and a pig received this fungus grown on maize, and a pig a culture of it grown on barley without any detrimental effects; and (*e*) two rabbits, a pig and a dog, took large amounts of fungus infected *Lolium temulentum* Linn (drabok) (see Leemann's article on this plant appearing elsewhere in this Journal) without suffering any detrimental effects.

Furthermore, each of two rabbits received 240 c.c. of pure Raulin's medium at the rate of 120 c.c. daily without suffering any ill effects.

The fact that the above-mentioned fungi grown on sterilized substrata did not produce any harmful effects upon the experimental animals, does not exclude the possibility of their producing toxic substances when growing together with other fungi and/or bacteria on certain substrata. This point is referred to under "Conditions affecting the toxicity of mouldy foodstuffs."

III.—DISCUSSION.

A.—Are Fungus-infected Foodstuffs Poisonous?

Although most varying results as to the toxic effects of mouldy foodstuffs were obtained by different investigators, it would appear from the literature that foodstuffs infected with the following fungi may at times be harmful to man and animal: "*Mucor mucedo*," "*Mucor racemosus*," "*Mucor stolonifer*," "*Mucor phycomyces*," "*Aspergillus glaucus*," "*Penicillium glaucum*," "*Oidium lactis*," "*Tilletia caries*," "*Ustilago carbo*," "*Ustilago longissima*," "*Ustilago echinata*," "*Puccinia graminis*," "*Puccinia coronata*," "*Puccinia arundinacea*," "*Uromyces apiculatus*," "*Polydesmus exitiosus*," "*Polythrincium trifolii*," "*Epichloë typhina*," "*Actinomyces thermophyles liquefaciens*," "*Endoconidium temulentum*," and "*Fusarium roseum*" Link. ("*Gibberella Saubenettii*").

"*Claviceps purpurea*" (ergot) and "*Claviceps paspali*" have been definitely proved poisonous on more than one occasion. In addition Mitchell (1918) and Theiler (1927 a) have proved beyond doubt that maize infected with *Diplodia zeae* (Schm.) Lev. is poisonous to cattle and sheep.

Recently specimens of fungus infected maize marketed for human consumption were received at Onderstepoort for investigation. These specimens contained up to 12 per cent. of mouldy mealies from which the following organisms were isolated by Drs. B. Doidge and A. C. Leemann of the Division of Botany, Pretoria: "*Penicillium*," "*Aspergillus*," "*Mucor*" and "*Fusarium*." All these fungi are considered harmful to health.

The quantities of these specimens of mealies and mealie meal submitted were too small to allow of feeding experiments being conducted.

The fact that *Diplodia zeae*, which was proved poisonous by Mitchell and Theiler, is frequently found infecting mealies, especially those grown in areas with a high rainfall, warrants special care in the utilisation of fungus-infected maize for household purposes and as a feed for stock.

B.—Symptoms of Poisoning.

The following symptoms have been attributed by different investigators to the ingestion of mouldy foodstuffs: inappetence, nausea, salivation, vomiting, constipation, diarrhoea, colic, tympanites, difficult deglutition, icterus, polyuria, cystitis, nephritis, dizziness, staggering, pronounced excitement, marked depression, hyperaesthesia, paralysis of the extremities, hindquarters, tongue, urinary bladder, ears and retina, complete general paralysis, pushing, turning somersaults, bellowing, trembling, convulsions, epileptiform spasms, profuse perspiration, imperceptible pulse, dyspnoea, rapid loss in condition, swelling of the eyelids, abortion, catarrh of the upper air-passages, urticaria, itching, conjunctivitis, vesicular eczema, fever, cyanosis and mummification and necrosis of the prominent parts of the body. The mortality is fairly high.

Apart from these symptoms the spores of rusts and smuts may when inhaled in large amounts cause severe irritation of the mucosa of the buccal cavity and upper air-passages, especially in the human being.

In addition to the above harmful effects attributed to fungi, Jármai (1925) produced visceral and articular gout in geese by force-feeding them with "*Penicillium glaucum*" infected maize.

C.—Post-mortem Lesions.

These are recorded as: gastro-enteritis with haemorrhages and erosions, accumulation of serum in the brain cavities and in the arachnoidal sac, hyperaemia and oedema of the brain and spinal chord, haemorrhagic fluid in the peritoneal cavity, cystitis, nephritis, peritonitis, acute yellow atrophy of the liver, bronchitis, haemorrhages in the serous membranes, swelling of the spleen, turbid swelling of the liver, kidneys and myocard and necrosis and mummification of extreme parts of the body.

In some cases the autopsy is negative.

From the above symptoms it would seem that some cases of so-called poisoning with mouldy foodstuffs were probably botulism and parabotulism.

D.—Conditions Affecting the Toxicity of Mouldy Foodstuffs.

It appears generally accepted that the mycelia and spores of fungi are harmless except for the irritation of mucous membranes caused by them when they are inhaled in large quantities. There are, however, a few exceptions, for example, "*Claviceps purpurea*" and "*Claviceps paspali*," the sclerotia of which are poisonous.

It is considered that the fungi attacking foodstuffs decompose certain constituents of the latter into poisonous substances. Theoretically there are two ways in which fungi could render the substrata toxic, namely, (*a*) by forming poison in their mycelia and spores and retaining it within their own structures, or secreting it into the substrata, or (*b*) causing the formation of poisonous substances by breaking up one or more constituent of the substrata. The latter point will be further elucidated under "The toxic constituents of mouldy foodstuffs."

The following factors may be, and probably are, concerned in the determination of the toxicity of fungus-infected foodstuffs :—

(1) More than one fungus may be necessary to cause the forming of poisonous substances in the substrata, or, a certain fungus may require the presence of some other definite fungus or fungi in order to liberate toxic substances in the substrata.

(2) Fungus-growth (one or more kinds) may have to be associated with the growth of some or other bacterium or bacteria.

(3) Another possibility is that the harmful substances liberated in the substrata by fungus- and bacterial-growth may in themselves be very slightly toxic and that, when present in the same substrata they may, through their synergistic action, become highly toxic.

(4) There is no reason why the different strains of the same fungus should not, as in the case of bacterial strains, vary to a considerable extent in their capacity of producing poisonous substances in the media on which they grow.

(5) The period of growth of the fungus, or of the fungus together with certain bacteria on the substrata may be a limiting factor in the production of toxic substances.

(6) Aeration, moisture content, reaction (whether acid, neutral or alkaline) and temperature of the substrata, most probably influence the liberation of poisonous ingredients.

(7) The constitution of the substrata plays, in some cases at least, an important role in the formation of certain harmful substances, for example, it was found that certain fungi produce more oxalic acid in the substrata when the latter contain calcium than when they are calcium-free. Furthermore, with regard to the production of oxalic acid in fungi cultures, it was found that the origin of the nitrogen present in the media was of great importance.

(8) The individual susceptibility of man and animal, apart from the fact that different classes of animals vary considerably in their susceptibility to poisoning with mouldy foodstuffs, undoubtedly is a factor concerned. Individual susceptibility may in some cases be explained by an inhibited function of the liver and kidneys (cirrhosis, degeneration) and an inflammatory condition of the gastro-intestinal tract at the time of poisoning.

(9) In addition to the above possibilities, which may explain the contradictory information with regard to poisoning with mouldy foodstuffs, I would like to mention that the different investigators, owing to incorrect identifications, may not have been working with the same kind of fungus in spite of the fact that it was recorded as one and the same kind.

E.—The Toxic Constituents of Mouldy Foodstuffs.

The toxic principles of *Claviceps purpura* (Fr.) Tul. (ergot) are accepted to be ergotoxine, ergotinine, tyramin and histamin, and Gieger (1932) succeeded in isolating a very poisonous alkaloid from "*Claviceps paspali*."

The following poisonous ingredients were, according to the literature quoted, isolated from fungus-infected foodstuffs : glucosidal and alkaloidal substances, biogenic amines (cholin), furfurol, acetic acid, formic acid, phlogosin-like substances, aldehydes, alcohol, acids, trimethylamin, tyramin and ergotin.

Brown (Editorial, 1916), who examined the root of *Mesembrianthemum Mahoni* N.E. Br. found that it contained a mould "*Rhopalocystis nigri*" (="*Aspergillus niger*"), which produced a large amount of oxalic acid when grown on sugar solution.

In the investigations of the toxic constituents of mouldy foodstuffs and artificial cultures of moulds, attention has almost exclusively been paid to the products of decomposition of the proteins present in the substrata. The three substances present in the substrata most likely to be attacked by the fungi are starches, proteins and fats. It seems quite feasible that non- or very slightly toxic substances will result from the decomposition of fats as these will in the presence of starches and proteins no doubt be very slightly attacked by most fungi.

It would appear that each and every case of poisoning with mouldy foodstuffs will have to be considered on its own merits with regard to the substances responsible for the poisoning. Mouldy foodstuffs of a high protein and low starch content will be liable to form toxic biogenic amines as products of decomposition of the proteins. These toxic biogenic amines may be cholin, acetylcholin, neurin, easily hydrolysable esters of cholin, trimethylamin, tyramin, etc.

On the other hand, starchy foodstuffs (cereals) low in protein will be liable to be decomposed by fungi into the following harmful substances: alcohol, acetaldehyde, ethylaldehyde, formaldehyde, furfurol, acetic acid, formic acid, oxalic acid, etc.

In mouldy foodstuffs containing both starches and proteins the above products of decomposition may by reason of their synergistic action become highly toxic. A further increase in toxicity may be effected by decomposition products caused by bacteria, which are frequently to be found in such foodstuffs.

Fresh hay poisoning is still an unsolved problem. The cumarin contained in fresh hay appears not to be directly concerned in the causation of poisoning.

F.—Immunity to Mouldy Foodstuffs.

(a) *Natural immunity.*

There appears to be an appreciable difference in the susceptibility of the different classes of animals to the effects of mouldy foodstuffs.

Ruminants (cattle and sheep) seem to be more susceptible than horses, mules, donkeys and pigs to "*Diplodia zeae*"-infected maize, whilst it would appear that as a rule pigs, horses, mules, dogs and man are much more susceptible than cattle, sheep, goats and poultry to fungus-infected foodstuffs.

It would thus seem impossible to lay down a general rule as to the relative susceptibility of the different classes of animals to fungus-infected foodstuffs.

(b) *Acquired immunity.*

The available information with regard to an acquired immunity to mouldy foodstuffs is far too incomplete to allow of any definite opinion being expressed The results of immunisation experiments with extracts of "*Aspergillu fumigatus*" were negative.

G.—Can Mouldy Foodstuffs be Utilised as Stock Feeds.

This is a question of enormous economic importance to stock owners. While foodstuffs infected with certain fungi may be harmless or even rendered more easily assimilable, owing to the breaking-up by these fungi of otherwise less digestible constituents, it would seem advisable to consider all fungus-infected foodstuffs detrimental to the health of man and animal. On the other hand, such foodstuffs, owing to the fact that they are never, or very rarely, acutely poisonous, may in many cases be utilised as a feed for certain classes of stock provided they do not form more than 10 per cent. of the total ration. However, before proceeding to use mouldy foodstuffs as a general feed, stock owners would be well advised to ascertain the harmfulness or toxicity of such foodstuffs by conducting preliminary feeding experiments on stock of low value. From the foregoing discussion it would appear that as a general rule care should be exercised in the feeding of mouldy foodstuffs to horses and pigs, while ruminants and fowls appear to be less susceptible. In the case of maize infected with *Diplodia zeae* the reverse appears to be true.

IV. SUMMARY.

(1) For all practical purposes fungus-infected foodstuffs must be considered poisonous until the contrary has been proved by extensive feeding experiments on the different classes of stock.

Diplodia zeae infected mealies have on several occasions been proved poisonous.

(2) It would appear that some cases of food poisoning in animals attributed to fungi were most probably cases of botulism and parabotulism.

(3) Conditions which probably play a role in the formation of toxic substances in mouldy foodstuffs, are discussed.

(4) Reference is made to the possible toxic ingredients of mouldy foodstuffs and more attention than has hitherto been the case, is paid to the possibility of decomposition products of carbohydrates being poisonous.

(5) The problem of fresh hay poisoning is still a mystery.

(6) More attention should be paid to the bacteria (and the products of decomposition caused by them) associated with fungal growth on foodstuffs

(7) There is an appreciable difference in the susceptibility of the different classes of stock to fungus infected foodstuffs.

(8) In the literature consulted no definite information with regard to the development of immunity to mouldy foodstuffs is available.

(9) It would appear that harmful mouldy foodstuffs could be fed with impunity to some classes of stock provided they do not constitute more than 10 per cent. of the total ration of such animals.

(10) It is generally accepted that the toxicity of *Lolium temulentum* Linn. (drabok, darnel) to man and animal is due to the grains being attacked by a fungus "*Endoconidium temulentum*"; the active principle is considered to be an alkaloid temulin, which is said to be absent in fungus-free darnel.

(11) Maize and bran very heavily infected with fungi were fed to fowls and rabbits, and also cultures of "*Fusarium moniliforme*," "*Fusarium moniliforme*" var. "*subglutinans*" and "*Fusarium graminearum*" Schwabbe grown on various substrata were fed and drenched to rabbits and pigs without having deleterious effects.

Large amounts of fungus-infected *Lolium temulentum* Linn. (drabok, darnel) were fed to two rabbits, a pig and a dog without these animals suffering any ill-effects.

V.—ACKNOWLEDGMENTS.

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ADDENDUM.

By Dr. DOIDGE, Principal Plant Pathologist, Division of Plant Industry, Pretoria.

221. *Fusarium graminearum* Schwabe.
222.
223. *Fusarium moniliforme* Sh.
224. var. *subglutinans* Wr. and Rkg.
225. *Fusarium moniliforme* Sh.

Strains 221–225 were isolated from mealie meal supplied by a miller at Bethal, O.F.S., to a firm in the Eastern Transvaal. Natives refused to eat this and said it made them sick. Sample was sent to us by Dr. Murray of the Health Department and under the microscope showed almost as many *Fusarium* spores as starch grains.

F. graminearum is the conidial stage of *Gibberella Saubinettii* and was referred to as *F. roseum* in some of the earlier publications.

284. *F. moniliforme* v. *subglutinans* from maize used for feeding animals at the Zoo: the animals afterwards suffered from paralysis.

(It is not possible that this maize was also infected with *Diplodia zeae*, and that we failed to isolate this fungus from the sample sent?)

550. *F. moniliforme*.—Isolated from green mealie purchased on Pretoria market, March, 1930. The grains were turning light brown and decaying in patches, and some showed a pink discolouration.

602. *Fusarium moniliforme* v. *subglutinans* from grains of maize from Klip River, sent from Pharmacology Department, Witwatersrand University. This is the organism which Prof. Watt worked with, and will probably be mentioned in his book.

622. *Fusarium graminearum* Schwabe. Isolated by Dr. Leemann from maize cob showing moulding and a pinkish discolouration; sent by Prof. Watt.

630. *F. graminearum* Schwabe. Isolated by Capt. J. McDonald, Kenya, organism frequently obtained from maize seeds showing no visible signs of disease.

631. *F. moniliforme* v. *subglutinans*. Isolated from maize by McDonald in Kenya.

632. *F. moniliforme* v. *subglutinans*, from maize seeds germinating on cob sent by Mansveld, Field Husbandry Section from Piet Retief. Isolated by Leemann.

665. *F. moniliforme* v. *subglutinans*, from maize plants from Kinross. Organism isolated from leaf bases, the upper nodes and leaf bases of these plants were badly collapsed.