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Cairo University Faculty of Veterinary Medicine, Giza, Egypt

Studies on the toxins of Aspergillus niger isolated from meat

S. H. KAMEL, M. REFAI and A. LOOT

Summary

Studies on the fungal flora of meat revealed that Aspergillus niger was the most common, followed by Penicillium species, Mucor, A. fumigatus, Rhizopus, Streptomyces, Pullularia, Cephalosporium, Alternaria, Scopulariopsis, A. flavus and Botrytis.

The production of toxins was detected by the chemical test (Shestak); by the skin test (Forgacs & Carll) and by strong fluorescence on exposure to U. V. light. LD_{50} in rats was 1.24 ml mycelial extract/100 g body weight; LD_{95} was 1.81 ml and 0.65 ml was the smallest dose that caused death of rats when injected intraperitoneally.

In dogs, the liver was the organ most commonly affected. It showed toxic changes varying from cloudy swelling to marked fatty metamorphosis. There were small haemorrhages in the brain and perivascular cuffs. The kidneys presented interstitial nephritis. In one dog there was endometrial cystic hyperplasia.

Introduction

Since the discovery of aflatoxins in England (ALLCROFT et al., 1961), several reports appeared in the literature concerning mycotoxins and their importance to man and animals. Apart from Aspergillus flavus, other species of Aspergilli can produce mycotoxins as A. fumigatus, A. niger, A. nidulans and A. glaucus (HANSSEN and HAGEDORN, 1969). Most of the mycotoxinproducers are moulds that belong to the class Deuteromycetes which are widely distributed saprophytes (FRANK, 1972). At present, about 250 fungi are known to produce toxins, among which 57 species of Penicilli, 34 Aspergilli and 18 Fusarium were described (MEYER and LEISTNER, 1969).

The present work is dealing with studies of the fungal flora of meat in slaughter houses, butcher's shops and the surroundings; moreover, the toxicity of Aspergillus niger, the most commonly isolated fungus, was investigated.

Materials and Methods

5622 swab-samples taken from the surface of meat and the surroundings were cultured on Sabouraud glucose agar and incubated at room temperature. The isolated fungi were identified according to their macro- and microscopic morphology.

For the preparation of toxins, A. niger was cultured on Sabouraud glucose broth in Roux-bottles at 25 °C for 2 weeks. The fungal mat was subjected to reflux extraction with ether, and the culture broth was filtered by EK Seitz-filter. Both the extract and culture filtrate were tested for the presence of toxins by ring-test (SHESTAK, 1960), dermal test in rabbit (FORGACS and CARLL, 1962) and by absorption of U. V. light (WOGAN, 1966).

For the estimation of the mortality dose response curve (BLISS, 1962), 7 groups, each of 10 rats, were injected intraperitoneally with increasing doses of A. niger extract (0.4 multiplied by 1.3, up to 1.8 ml/100 g body weight). For the statistical analysis the experiment was repeated again on 6 groups of 10 animals and on 7 groups of 6 rats. In each experiment non-injected control group was used.

The pathogenicity of A. niger to 4 dogs, of which 2 were females, was studied by feeding mouldy bread twice daily for two weeks, and to 8 dogs by i. p. and i. v. injection of EK Seitz-filtered broth culture. The animals were killed after 2 weeks and the livers, hearts, lungs, kidneys and brains were examined histopathologically.

Results

1. *Fungal flora of meat and the surroundings*

3127 strains of moulds were isolated which could be identified as Aspergillus niger (1313 strains), Penicillium spp. (841), Mucor (254), Aspergillus fumigatus (125), Rhizopus (117), Streptomyces (113), Pullularia (77), Cephalosporium (71), Alternaria (66), Scopulariopsis (53), Aspergillus flavus (66) and Botrytis (47). The same fungi were isolated from the air, walls and floors of the slaughter houses **(Fig. 1).**

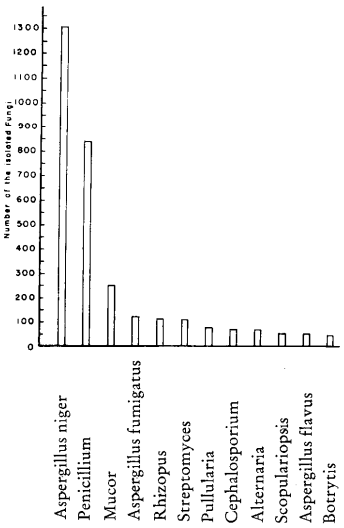

Fig. 1: Fungi isolated from meat and surroundings

2. *Detection of Aspergillus niger toxins*

a. Chemical test: The presence of toxins in the mycelial extract and cultural filtrate was confirmed by the formation of a white ring at the junction of the ether and the mixture of extract or filtrate with 2 N Sodium hydroxide solution.

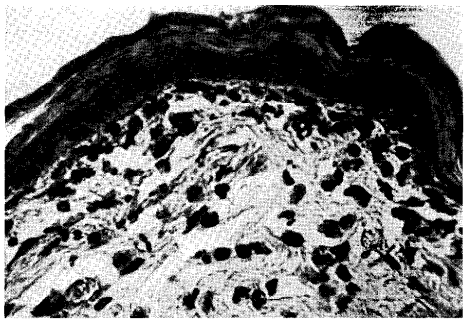

Fig. 2: Skin of rabbit showing necrotic changes and neutrophilic infiltration

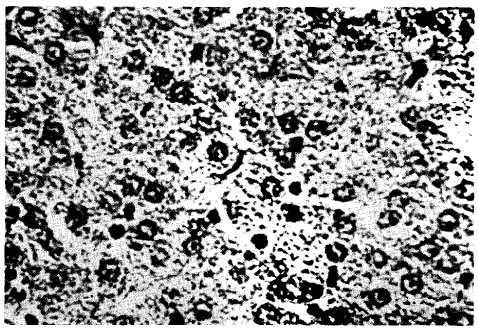

Fig. 4: Cloudy swelling in liver of dog

b. Absorption of U. V. light: Both filtrate and extract showed strong fluorescence when exposed to U. V. light.

c. Dermal test: The application of the mycelial extract in oil to the skin of rabbit caused mild acute dermatitis. Histologically there was a necrotic debris around the external epithelial surface and thinning of the viable cell layers of the epithelium. Just beneath the stratum germinativum polymorphonuclear neutrophils have accumulated. All blood capillaries and larger venules were congested and a perivascular cuff of neutrophils and mononuclear leukocytes was prominent in one area of the dermis (**Fig. 2**).

3. The mortality dose response curve

It was found that 0.65 ml of the extract/100 g body weight was the smallest dose that caused death of 10 % of the rats in 24 hours, when injected intraperitoneally. The mortality percentage increased in accordance with the injected dose. All rats died when 1.8—2.4 ml/100 body weight were injected. The slope of linear regression of the dose mortality curve of rats proved to be very highly significant, 2 P = 0.001. Regression equation to the straight line representing the relationship between % mortality and the magnitude of dosage, showed the following lethal doses (**Fig. 3**):

LD_{95} = 1.81130
LD_{50} = 1.24120
LD_{10} = 0.73444

The dead rats presented cyanosis of the nose, limbs and mucous membranes. The liver, spleen and kidneys were highly congested.

4. *Pathogenicity of A. niger to dogs*

(a) The 4 dogs fed with mouldy bread and killed after 2 weeks showed icterus of mucous and serous membranes, yellowish to orange discoloration of the liver, enlargement and distension of the gall bladder and occasional haemorrhage into the gastro-intestinal tract. In the 2 females the uterus was inflammed.

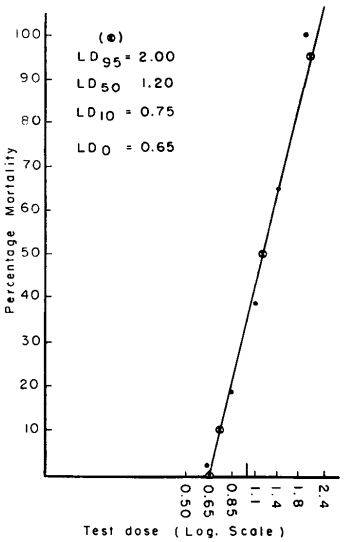

Fig. 3: Mortality % in response to lethal dose

Histopathology: marked toxic tissue changes were observed. The changes in the liver varied from cloudy swelling with acidophilic and granular hepatocyte degeneration to marked fatty metamorphosis in the portal peripheral regions of the liver lobules (**Figs. 4, 5**). Microscopic haemorrhages confined to the gray matter were present in the brain tissues of two dogs. The third dog had prominent perivascular cuffs of lymphocytes in the cerebral white matter (**Fig. 6**). The fourth dog had mild gliosis of the caudate nucleus. The kidneys showed interstitial nephritis.

(b) The i. p. or i. v. injection of 1ml/kg body weight of the cultural filtrate of A. niger caused the same p. m. findings, as in the previous experiment, in all 8 dogs injected. Histopathologic findings were confined to the liver. The changes consisted of cloudy swelling with obliteration of hepatic sinusoids and early degenerative change in hepatocytes with acidophilic and granular degeneration. The kidneys showed interstitial nephritis. One dog only had cystic hyperplasia of the uterine endometrium.

Discussion

The studies of the fungal flora of meat revealed the presence of large number of various types of moulds on the surface of meat. Some of the isolated fungi are known to be pathogenic for man and animals, e. g. Aspergillus fumigatus, the cause of pulmonary aspergillosis in man and the brooder pneu-

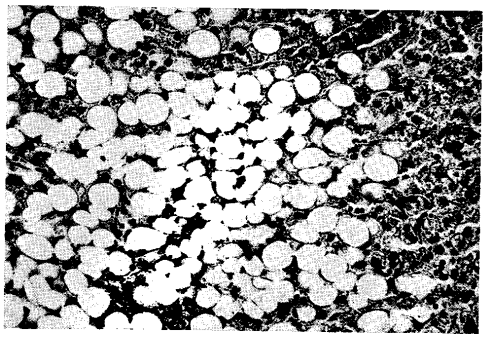

Fig. 5: Fatty metamorphosis in liver of dog

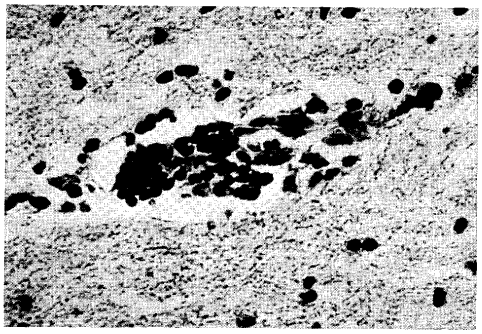

Fig. 6: Perivascular cuffs in brain of dog

nia in checkens (PENA, 1970). Aspergillus flavus is known to produce hepatotoxic and carcinogenic metabolites which are designated as aflatoxins (ALLCROFT et al. 1961, FRANK 1972 and BÖSENBERG 1972). Little informations are available with regard to A. niger toxins. It was listed by BORKER et al. (1960) among other types of Aspergilli implicated in mycotoxicoses. Furthermore BURZYNSKA (1971) mentioned that extracts from cultures of A. flavus, A. niger and a Penicillium sp. were highly toxic to ducklings.

In the present work, the presence of toxins in the A. niger culture filtrate and mycelial extract was proved by chemical test, strong fluorescence when exposed to U. V. light, as well as by dermal test in rabbit. The rats were found to be susceptible to the toxins in dogs, the liver was the organ mostly affected and presented classical toxic changes. This is characteristic to aflatoxin which is known to cause acute inflammatory changes in the liver (BÖSENBERG, 1972).

In this respect ARMBRECHT et al. (1971) administered aflatoxin orally to dogs, where they noted bile duct proliferation, bile pigment accumulation in the portal areas and multiple vascular channels around the central and portal veins.

Even monkeys, orally administered with aflatoxins, showed extensive hemorrhagic necrosis of the liver (DEO et al., 1970), or necrosis with midzonal fatty changes (ALPERT et al., 1970). The small haemorrhages seen in the brain in the present work are probably a reflection of recent vascular damage. The perivascular cuffs are common C. N. S. changes in toxic and infectious processes as a response of neural damages. The significance of gliossi of caudate nucleus is not yet known. Most of the dogs showed interstitial nephritis, meanwhile kidney damage was reported in pigs fed on barley infected with different moulds (BUCKLEY, 1971).

From the aforementioned results and discussion it can be concluded that Aspergillus niger is able to produce toxins that cause death of rats, inflammation of the skin of rabbits and toxic changes in the internal organs of dogs. It is hoped, therefore, that this work may throw some light on the importance and danger of contamination of food and feeding stuffs to man and animals.

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Address for reprints:

Prof. Dr. M. REFAI,
Food Contamination Project,
FAO (UNDP),
Nairobi, Kenya.