BIOLOGICAL ACTION OF FLUORESCENT SUBSTANCES EXCRETED FROM THE FUNGUS Aspergillus niger

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UDC 582.282.123.4:576.8.095.14

Fluorescent substances possessing toxic hepatotropic activity (in experiments on rats) are excreted by the fungus Aspergillus niger strain No. ÉU119. If A. niger is used as the source of enzyme preparations for use in the food industry, these products must be purified to remove fluorescent contaminants.

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Considerable attention has recently been paid in the literature to toxic metabolic products of lower fungi of the genus Aspergillus. This is because, besides their marked toxicity, some of them (such as the aflatoxins) possess characteristic hepatotropic activity and high carcinogenicity [2, 3, 5, 8]. The detection of these substances is based on their property of fluorescence in ultraviolet light [4, 6, 7, 9].

Investigations in the writers' laboratory have shown that cultures of certain strains of the fungi Aspergillus niger and Aspergillus awamori contain fluorescent compounds in their mycelium, which, however, differ in their physicochemical properties from the known aflatoxins [1].

In the investigation described below the action of purified fluorescent compounds isolated from Aspergillus niger strain No. ÉU119 on animals was studied. A culture of this fungus is used to obtain citric acid and enzyme preparations for the food industry, and it is thus important to detect any potentially toxic impurities likely to enter the human body.

EXPERIMENTAL METHOD

Fluorescent substances were obtained as follows. The dried mycelium of the fungus was placed in bottles with ground glass stoppers, covered with 10 volumes of methanol, and agitated on a shaker for 24 h. The methanol extract was then separated by filtration and diluted with water in the ratio of 3:2. The dilute extract was transferred to a separating funnel, an equal volume of chloroform was added, and the mixture was shaken for 2-3 min. The chloroform layer (the bottom layer) was poured off into evaporating dishes. This procedure was repeated three times and all the chloroform extracts were pooled. The chloroform was then evaporated off on a water bath until the volume of residual extract was 2-3 ml. The extract was then subjected to preparative thin-layer chromatography. Fractionation was carried out in a chloroform – methanol system (97:3 by volume). After the solvent front had reached a point 9-10 cm from the starting point, the discs were removed from the vessel and dried in the air. Regions of the migrating bands with blue and violet fluorescence were noted in ultraviolet light on the discs. The silica gel in these regions was cleaned, placed in a funnel with a filter, and washed with a large volume of chloroform, which was collected in an evaporating dish. The chloroform was evaporated off on a water bath to dryness. The dry yellow residue was subjected to control chromatography. One stain with blue fluorescence, having an Rf value of 0.45, and another with violet fluorescence and Rf = 0.28 were observed in the course of the determination.

The residue thus obtained was fed to albino rats in a single dose in sunflower oil. The animals were kept under observation for 3-8 days, their body weight, behavior, appetite, and the function of their gastro-

Laboratory of Investigation of Enzyme Preparations, Institute of Nutrition, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 69, No. 2, pp. 44-46, February, 1970. Original article submitted July 17, 1969.

intestinal tract were recorded, a blood count was taken, and the activity of acetylcholine esterase, and alanine and asparagine aminotransferase in the blood was determined. After 2-8 days the rats were decapitated in groups of 3 or 4 and the internal organs were investigated histologically. Pieces of the organs were fixed in calcium-formol and in Carnoy's fluid. Sections were stained with hematoxylin-eosin, azure II-eosin, and by Mallory's method. Lipids were detected by a mixture of Sudans II and III by Goldman's method, polysaccharides by McManus's method, and nucleic acids by the methods of Brachet and Feulgen.

EXPERIMENTAL RESULTS

No visible changes were found at autopsy on the 3rd-8th day after administration of the purified compounds in a dose of between 6 and 78 mg to animals weighing 200 g. When the dose was increased to 160 mg, on the 4th day of the experiment some rats showed hemorrhages into the subcutaneous cellular tissues. The relative weight of the kidneys was also reduced in the animals of this group (1.11±0.01 g in the experimental series, 1.32±0.01 g in the control).

Histological examination of the internal organs showed structural changes which were particularly marked in the liver. With a background of venous congestion, the liver parenchyma consisted everywhere of enlarged, pale cells, with a thickened, condensed membrane giving a PAS-positive reaction. The cytoplasm had the appearance of optically empty cavities, irregular in shape, without sharply defined borders, and connected by areas of intact, dense cytoplasm (Fig. 1a, c). The content of protein and RNA in the cells was considerably reduced, except in dense areas of cytoplasm between the cavities and around the nuclei. Glycogen and lipids were absent. Many cells contained two nuclei. As well as normal nuclei, some were in a state of lysis. Approximately one-third of the cells contained hyperchromic nuclei, with large, pyroninophilic granules inside them. Differentiation with ribonuclease did not cause these grandules to lose their pyroninophilia. Normal nuclei stained green by Brachet's method, while hyperchromic nuclei stained dark crimson. The nucleoli were RNA-positive, but Feulgen-negative, and for this reason the nucleolar DNA could not be seen in the hyperchromic cells. The nuclear membrane was slightly undulating in appearance. In the periportal zone of the lobules, small foci of proliferation of bile duct epithelium were observed (Fig. 1b).

Venous congestion and foci of degeneration were observed in the epithelium of the convoluted tubules of the kidneys. In other organs, slight venous congestion was present, with selling of the endothelium in places and edema of the structural elements of the adventitia.

The fluorescent substances present in the mycelium of A. niger thus caused structural changes in the internal organs and possessed well-marked hepatotropism.

Culinary citric acid and a preparation of the enzyme peptinase obtained from a culture of the fungus A. niger and intended for use in the food industry were tested for their content of fluorescent impurities. No such impurities were found in the citric acid, but the peptinase preparation contained two fluorescent fractions, like the mycelium of the fungus.

In experiments on rats, observations were made on the histological structure of the internal organs following single and prolonged administration of this enzyme preparation. When a single dose of the preparation was given the total quantity of fluorescent substances contained in it was only 4 mg per rat (5 g/2 kg weight of the preparation). During prolonged administration of the preparation to animals of the two groups, in doses of 50 and 250 mg/kg for 12 and 6 months, the total quantity of fluorescent fractions received by each rat was about 20 and 40 mg, respectively.

Histological examination of the organs of rats sacrificed 7 days after receiving a single dose of the enzyme preparation showed pathological changes in the structure of the internal organs, especially the liver and kidneys. The liver showed stasis of blood in the lumen of the sinusoidal capillaries and cloudy swelling of the cytoplasm of the hepatocytes (pale cells) against the background of hyperplasia of the reticular Kupffer cells and focal collections of monocytes. Lysis of the liver cells was observed where these monocytes accumulated.

In contrast to this, after administration of a single dose of the same quantity of enzyme preparation from which all fluorescent substances had been removed by repeated methanol extraction, no such changes were observed in the liver.

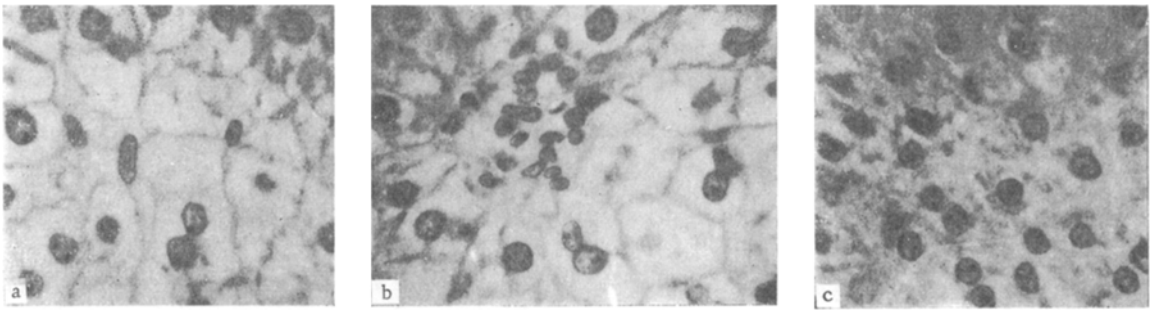

Fig. 1. Changes in liver of rats after oral administration of a single dose of fluorescent substances from Aspergillus niger: a) cloudy swelling (of the balloon type) of hepatocytes; b) cloudy swelling of hepatocytes, proliferation of bile duct epithelium; c) control. In a and c, staining by Brachet's method; in b, staining with hematoxylin-eosin. 900×.

In rats which received the enzyme preparation over a long period of time, stasis of blood in the venous and capillary systems was found in the internal organs. Mural thrombi were present in the lumen of certain central veins of the liver, and focal hemorrhages were seen in its parenchyma. Degenerative changes of the same character as in the preceding experiments developed in the liver cells. Foci of necrobiosis of the hepatocytes were replaced by tissue consisting of reticular cells and histiocytes. Fibrosis also affected the tissues of the portal interstices. The kidneys showed focal hemorrhages and cloudy swelling of the epithelium of the convoluted tubules.

The results of these experiments indicate that fluorescent substances contained in the mycelium of the fungus Aspergillus niger strain No. ÉU119 have a toxic action on experimental animals, producing the most marked changes in the liver. In acute experiments the pattern of the pathological changes is characterized by the appearance of "pale" cells.

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