Nephro- and Hepatotoxicity for Mice and Chicks in *Penicillium piceum* Contaminated Food[1]

Mold contaminated foodstuffs are known to be toxic to animals. It has been reported earlier that common food contaminants like *Aspergillus flavus*[2] and *Penicillium rubrum*[3] produce toxins. The toxins elaborated by *A. flavus* called aflatoxins[4], and by *P. rubrum* known as rubratoxins[5], are found to be hepatotoxins[6]. The carcinogenic nature of aflatoxins has been well established[7]. The present report deals with the production of toxins by *P. piceum*, normally found in contaminated food. The toxins elaborated by *P. piceum* have been interestingly found to be both hepatotoxic and nephrotoxic.

Materials and methods. The organism *P. piceum* was grown at room temperature in Capek's medium in a Haffkin's flask for 20 days. The production of the toxin(s) was examined first in the culture filtrate. 2 l of the culture filtrate was filtered through cotton wool, then in a Seitz filter and finally concentrated to about 1/20 of its original volume under vacuum. 100 ml of this concentrate was extracted with double the volume of chloroform or ether for 1 h in a reciprocating shaker. The chloroform layer or the ether layer was separated from the aqueous layer. The solvents were removed by evaporation to absolute dryness and the residues were finally taken up in 100 ml of water.

200 g of sterilized commercial diet was moistened with 40 ml water, inoculated with 5 ml spore suspension (4×10^8 spores per ml) of *P. piceum* and incubated at room temperature for 18 to 20 days. The diet thus contaminated was sterilized by autoclaving (1.67 atm pressure for 10 min), dried and finely powdered. The experimental mice (Swiss albino weighing 20–25 g) were fed 5 g of this diet or uninfected diet mixed with 1 ml of the crude concentrate or its fractions for the whole day. The control mice received the uninfected diet. At the end of the experimental period (2–4 weeks) the animals were sacrificed and portions of liver and kindey were processed for pathological examination.

1-day-old chicks (white Leghorn) were fed once a day, with the culture filtrate or the various fractions. After 10 days, the surviving birds were sacrificed and the liver removed for pathological examination.

Results and discussion. 100% mortality was observed in chicks within 2 to 3 feedings of the culture filtrate or the aqueous solution of the ether fraction. However, with the other fractions mentioned in the procedure, no mortality was observed even when fed for 7 days. All the control birds were active and showed normal liver picture. Chicks fed with the various fractions of the culture filtrate showed granularity and vacuolation of the cytoplasm, mild dilatations of the sinusoids and slight necrosis in their liver cells.

It was interesting to note that there was no mortality in the case of mice. Figure 1 represents the section of a normal liver. Liver sections of mice fed with the infected diet, culture filtrate and the aqueous solution of the chloroform fraction, showed areas of mild fatty changes and areas of diffused liver cell necrosis with presence of binucleated liver cells, indicative of degeneration. Diffussed necrosis of the liver cells around the periphery can be seen in Figure 2. Sparse round cell infiltration and mild proliferation of the bile duct are the other observations noted in some cases.

A normal mouse kidney section is shown in Figure 3. The kidney sections of animals reared on 1. the infected diet, 2. the culture filtrate alone, and 3. the aqueous solution of the chloroform fraction showed round cell infiltration, cloudy swelling of the tubules and degeneration of the distal tubules and cells. Accumulation of the lymphocytes and focal nephritis around blood vessels can be seen in Figure 4. It was found that mice fed with other fractions showed some of these changes, but only to a very mild degree.

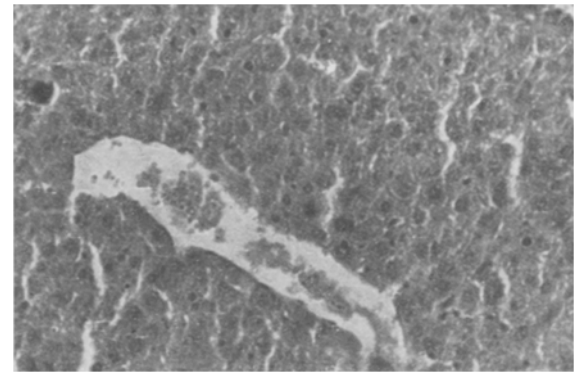

Fig. 1. Section of a normal mouse liver. ×400.

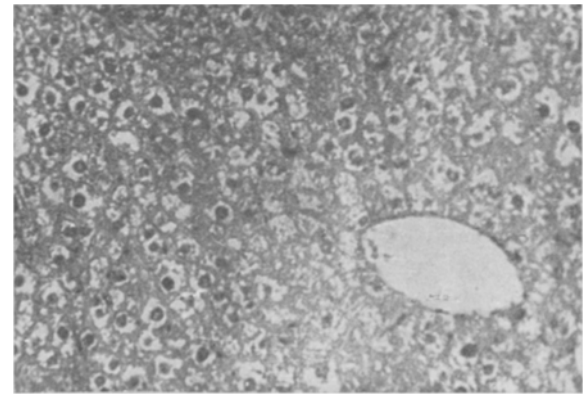

Fig. 2. Section of mouse liver showing diffused necrosis around the periphery. ×100.

The pathological lesions described above clearly indicate that the fungus *P. piceum* produces both hepatotoxins and nephrotoxins. It is well known that oxalic acid and citrinin are nephrotoxic[8]. Hence, it was our interest to see whether these two compounds are produced by *P. piceum*. Ether extraction was carried out to remove any oxalic acid, if present. It is interesting to note that the aqueous layer left after ether extraction was found to be toxic to 1-day-old chicks. Mild nephrotoxic symptoms were observed in the case of mice, too. The absence of

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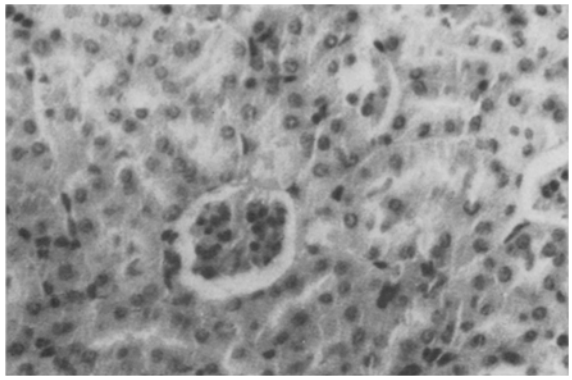

Fig. 3. Section of a normal mouse kidney. ×400.

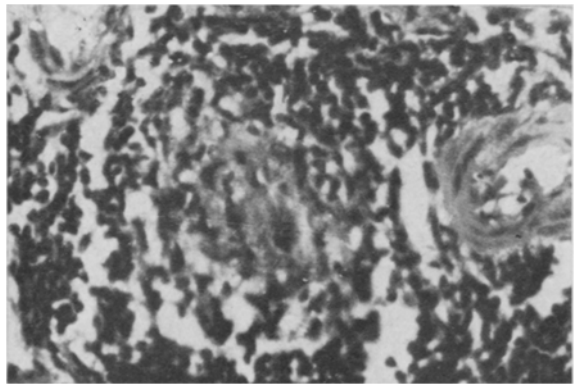

Fig. 4. Sextion of mouse kidney indicating focal accumulation of lymphocytes and focal nephritis. ×400.

citrinin was indicated by the chloroform extraction of the culture filtrate and subsequent co-chromatography with pure citrinin.

The pathological changes observed in our studies on kidney indicate glomerulonephritis, tending towards glomerulosclerosis[9,10]. Glomerulonephritis is understood to arise secondary to specific streptococcus infection of the respiratory tract or the skin[9,11,12]. Probably, our findings may be the first to indicate that the incidence of glomerulonephritis may be due to a primary effect of consuming contaminated toxic food. The isolation and characterization of the toxins are under way.

Zusammenfassung. Diäten, die mit Kulturen oder Kulturfiltraten von *Penicillium piceum* kontaminiert waren, erwiesen sich an Eintagsküken als strak toxisch und verursachten bei Mäusen ausgeprägte Nieren- und Leberschädigungen. Um eventuell vorhandene Oxalsäure und Citrinin, die nephrotoxisch wirken, zu eliminieren, wurde das Kulturfiltrat mit Äther bzw. Chloroform extrahiert. Die wässrigen Lösungen des Ätherextrakts waren bei Küken stark allgemein-toxisch, während bei Mäusen nur die wässrige Lösung des Chloroformextrakts diffuse Leberzellnekrose sowie Nierenveränderungen im Sinne einer Nephritis verursachte.

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Effect of Sulpiride on Oxygen Uptake by Rat's Brain Tissue in vitro

Sulpiride, a psychoactive drug, is a heterocyclic derivate (N(1-ethyl-2-pyrrolidinil-methyl)2-methoxy-5-sulfamoil benzamide) and was introduced by JUSTIN-BEÇANSON et al.[1]. Its pharmacological properties were studied by LAVILLE[2], ERNST and CHOTEAU[3] and LELIÉVRE[4].

It is known that some psychoactive drugs inhibit the oxygen uptake by the brain tissue in vitro and such effect is more evident when the tissue respiration has been stimulated by high potassium concentrations[5,6] or by deficit of calcium in the medium[7].

The purpose of this paper is to study the influence of sulpiride in the oxygen uptake in vitro by brain homogenates and by brain slices with low and high potassium concentrations and in absence of calcium in the medium.

Material and methods. Adult male albino rats were used to prepare brain slices, as described by MCILWAIN and BUDDLE[8], and brain homogenates. The slices were incubated in Krebs-Ringer phosphate medium (pH 7.4) which contained 10 m*M* glucose and 5 m*M* or 100 m*M* potassium or using the same Krebs-Ringer containing 5 m*M* potassium but without calcium. Whole brain homogenates were prepared at 2–4 °C in a Potter-Elvehjen homogenizer mixing 1 g of tissue with 9 ml of a solution containing 0.25 *M* sucrose and 0.1 *M* phosphate buffer (pH 7.4).

Oxygen consumption was determined by direct manometric technique[9] using a conventional Warburg apparatus with air as gas phase. Student's *t*-test was applied for statistical analysis[10].

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