The figure shows the mean radioactivity (cpm $\times 10^{-3}$) in 1 ml of bathing fluid withdrawn from explants after 20 h incubation. Absolute values vary between experiments because 400 µCi of radiocalcium per animal was given i.v. in the 1st and only 200 µCi in the 2nd, while in the 3rd 400 µCi was given i.p., which improved labelling of the skeleton. The use of an organic solvent proved unnecessary. Calcitonin eardrops significantly depressed parathyroid hormone-stimulated mobilisation of calcium from the earbones in tissue culture, whether it was dissolved in DMSO or in water containing a detergent, and whether the entire ossicular chain was examined or the explanted stapes alone, which is furthest from the presumed sites of diffusion through the ear drum and external meatus. Measurements (not shown) of radioactivity released after 40 and 60 h (with changes of medium every 20 h) showed undiminished active osteolysis for at least 60 h in all explants from PTH-treated animals; release from the ossicles of CT-treated ears remained lower than controls throughout this time.

No calcitonin effect was observed in the guinea-pigs to which it was given in propylene carbonate. This was probably due to limited solubility of the peptide in this solvent, since a saturated solution was subsequently found to contain only 53 µg/ml (Lowry determination, with standards containing matching dilutions of PC).

We believe these experiments provide the first evidence of effective transdermal administration of a peptide hormone, although oxytocin is well known to cross the buccal mucosa and vasopressin is commonly given intranasally. In view of the fact that calcitonin in eardrops inhibited PTH-stimulated bone resorption at least as effectively as when it was added to resorbing bone in vitro[10], a limited therapeutic trial seems desirable to determine whether such ear drops can affect the progression of clinical otosclerosis. It may also prove possible to obtain beneficial effects by direct transepithelial absorption of calcitonin in other localized pathological processes in bone (e.g. peridontal resorption and the osteoporosis which commonly occurs around arthritic joints).

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Preliminary report on toxigenic fungal isolates of *Aspergillus niger* in market foods and foodstuffs

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Summary. 14 out of 22 fungal isolates of *A. niger* from market foods and foodstuffs were toxigenic to weanling rats. Approximately $^2/_3$ of these cultures were highly toxigenic. Histopathologic changes were observed mostly in kidney with necrosis of the tubular epithelium and to a less extent in liver with centrolobular necorsis of the hepatic cells.

Market foods and foodstuffs may be highly contaminated with storage fungi, included species of *Aspergillus, Penicillium, Fusarium* and *Rhizopus*[2-4]. Occurrence of mycotoxins produced by these fungi has been well recognized in the past decade[5,6] and mycotoxicoses have been reported in many animal species[7,8] and man[9,10]. However, the need for the mycotoxicological studies is increasing and the emphasis must be placed on human health. The extent of mycotoxin hazard to man can be better assessed, if the identity and the toxigenicity of the fungi involved is known. This report presents preliminary results of the toxigenicity tests of the most common contaminating fungal isolates of *A. niger* species in weanling rats.

Materials and methods. 22 fungal isolates of *A. niger* species came from a screening program in which fungi were routinely isolated from a variety of market foods and foodstuffs in Bangkok (1975), identified and tested for toxigenicity in weanling rats[3]. The fungi were grown in 2.8-l Fernbach flasks on sterile glutinous rice (250 g) and water (200 g) for 14 to 20 days at 25±1 °C. Moldy substrates were extracted 3 times with 750 ml of chloroform or methylene chloride:methanol (97:3, v:v), filtered and evaporated in vacuum rotary evaporator. The oily residue was poured into cooled swirling petroleum ether and filtered to harvest a precipitate (petroleum ether insoluble, PEI). The filtrate was evaporated to remove all petroleum ether to obtain oily residue (petroleum ether soluble, PES)[11]. Aflatoxins were determined according to a modified method described by Eppley[12]. Bioassays of PEI and PES were performed in groups of 3–5 weanling female rats (35–45 g b.wt) of Fischer derived strain, Animal Production Center, Faculty of Science, Mahidol University, Bangkok. PEI was administered i.p. in DMSO (0.05–0.1 ml) at the doses equivalent to 25.0 g and 12.5 g moldy rice. PES was administered po as oily residue at the maximum dose of 1.0 ml/rat. Controls consisting of PEI in DMSO and PES of non-inoculated glutinous rice were included in all bioassays. The duration of all toxicity tests was 7 days. A necropsy was performed on all animals sacrificed at 7 days or found dead, and their tissues fixed in 10% buffered neutral formalin. Paraffin sections were stained with hematoxylin and eosin.

Results. Results of bioassays revealed that a total of 14 out of 22 fungal isolates (63.6%) were toxic to weanling rats (table). The PEI produced by 13 out of 22 fungal isolates (59.1%) were toxic and the most toxic PEI was produced by *A. niger* (AN-058-75) from rice. Of 22 fungal isolates, only 6 PES (27.3%) were toxic to weanling rats and the most toxic PES was produced by *A. niger* (AN-004-75) from rice.

Toxicity of fungal extracts produced by various fungal isolates of *A. niger* to weanling female rats (35–45 g b.wt)

PS culture number	Food source	Mortality* PEI (25.0 g moldy rice) Dose (mg/kg b.wt)	Dead per tested	PEI (12.5 g moldy rice) Dose (mg/kg b.wt)	Dead per tested	PES (165.0 g moldy rice) Dose (ml/rat)	Dead per tested	Aflatoxin B_1** (ppb)
AN-004-75	Rice	1000	4/5	500	0/5	0.33	3/3	ND
AN-008-75	Rice flour	750	5/5	375	5/5	0.43	0/3	12
AN-010-75	Mung bean	240	0/3	120	0/3	0.90	3/3	ND
AN-012-75	Corn	675	5/5	338	5/5	0.80	0/3	ND
AN-022-75	Glutinous rice	556	3/3	278	3/3	0.40	0/3	34
AN-030-75	Mung bean	788	5/5	394	5/5	1.00	3/3	ND
AN-051-75	Roast peanut	513	5/5	257	5/5	1.00	3/3	ND
AN-052-75	Roast peanut	613	5/5	307	5/5	1.00	0/3	ND
AN-058-75	Rice	271	3/3	136	1/3	0.80	0/3	ND
AN-064-75	Soybean	725	5/5	363	5/5	0.80	3/3	ND
AN-068-75	Roast peanut	495	3/3	248	1/3	0.75	3/3	ND
AN-082-75	Roast peanut	750	5/5	375	5/5	1.00	0/3	ND
AN-A26-75	Raw peanut	390	3/3	195	3/3	0.60	0/3	ND
AN-A30-75	Raw peanut	700	3/3	350	0/3	1.00	0/3	ND

*PEI = petroleum ether insoluble fraction, dose equivalent to 25.0 g or 12.5 g moldy rice that inoculated with various fungal isolates of *A. niger* for 14 to 21 days at 25±1 °C. PES = petroleum ether soluble fraction, dose equivalent to 165.0 g moldy rice (except AN-051-75, dose equivalent to only 100.0 g moldy rice). **Aflatoxin B_1 content represents in µg/kg moldy rice. ND = non detectable.

In addition, a total of 5 out of 14 toxic fungal isolates (35.7%) produced toxic compounds presented in both PEI and PES. It was found that 2 fungal isolates of *A. niger* (AN-008-75 and AN-022-75) were capable of producing aflatoxin B_1 at levels of 12 and 34 ppb, and only trace amounts of aflatoxin B_2.

After administration of toxic PEI and PES, most of the rats died within 1–2 days except those rats treated with PEI produced by *A. niger* (AN-051-75) which died within a few h. Histopathologic changes were observed mostly in the kidney (85.7%) and liver (28.6%). These changes revealed necrosis of the tubular epithelium (78.6%), glomerular nephrosis (7.1%) and marked dilatation of the convoluted tubules (7.1%) of kidney. Minor histopathologic changes were observed in liver of the rats treated with PEI and revealed acidophilic degeneration, irregularity in nuclear sizes, necrosis of the bile duct cells and an increase in mitotic figures. However, severe centrolobular necrosis and midzonal hydropic degeneration of the hepatic cells of livers in the rats treated with PES produced by *A. niger* (AN-010-75) were observed.

Discussion. Fungal extracts from approximately $^2/_3$ of the fungal isolates of *A. niger* used in this study were moderately to highly toxic to weanling rats. Most of PEI produced by fungal isolates of *A. niger* caused necrosis of the tubular epithelium of kidney. It suggests that these toxic compounds in PEI are mostly nephrotoxins. Shank et al.[4] found that 4 out of 14 fungal isolates of *A. niger* (28.6%) from market foods and foodstuffs in Thailand were toxic to weanling rats. These fungi were subsequently reevaluated for the toxigenicity and 25 out of 33 fungal isolates (75.8%) were moderately to highly toxic to weanling rats. Some fungal extracts also caused necrosis of the tubular epithelium of kidney[13]. Thus, our data are similar to that of Shank et al.[4] and Angsubhakorn et al.[13]. As regards the toxicity of the fungal extracts, it is not known whether or not there is present any of the known toxic substances. However, 2 fungal isolates were capable of producing small amount of aflatoxin B_1 at the levels of 12 and 34 ppb, and these levels at the dose of PEI administered are lower than the lethal dose of aflatoxin B_1 in the weanling rat[8]. It might not be enough to cause death to the rats. Oxalate is another toxic compound produced by certain strains of *A. niger*[14]. In this study, oxalate content was highest at 170 µg/100 mg PEI produced by fungal isolate of *A. niger* (AN-022-75) and at the dose of PEI administered to the rats, it is much lower than in LD_{50} of potassium oxalate 196.0 mg/kg b.wt (unpublished data). Furthermore, it is very difficult to compare the toxicity of PEI to malformin C, a cyclic pentapeptides produced by fungal isolate of *A. niger*[15] because the information of the mechanism of toxicity is still lacking. Therefore, additional work appears warranted on the isolation, purification and elucidation of the structures and toxicity of the pure toxic compounds from these fungal extracts.

Our present results show that fungal isolates of *A. niger* capable of producing toxins can be isolated in Thailand from market foods and foodstuffs intended for human consumption. This finding may indicate potential problems of considerable importance to public health.

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