ration was offered to the animals. Animals of group 1 received the conventional concentrate mixture, whereas in group II 25% of the DCP requirement was met by water-treated Mahua cake. Animals of group III and IV received 50% and 75% of their DCP requirement through Mahua cake, respectively. No group with untreated Mahua cake was included in the experiment as animals refuse to accept it due to its extremely bitter taste.

Results and discussion: The chemical composition of Mahua cake is shown in table 1. Within 1 h of the treatment of Mahua cake with ordinary water at room temperature, about 80% of the saponins were removed (table 2). No additional advantage was observed by treating the cake either by hot water or alkali. The foamy material developed after aqueous sodium hydroxide treatment adhered more intimately with solubilized cake and with gradual lapse of time it was more difficult to remove saponins; which explains the peculiar nature of NaOH treatment (table 2).

The cake was palatable to the animals after treatment with water. The gain in the body weights of the animals in all the 4 treatments did not differ significantly (table 3). The daily growth rate in the experimental animals were comparable to earlier reports[5,6]. It is concluded that Mahua cake after proper treatment for removing saponins may possibly be used as a cattle feed in limited quantity.

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Transdermal uptake of a peptide hormone: Inhibition by calcitonin eardrops of induced osteolysis in guinea-pig ossicles

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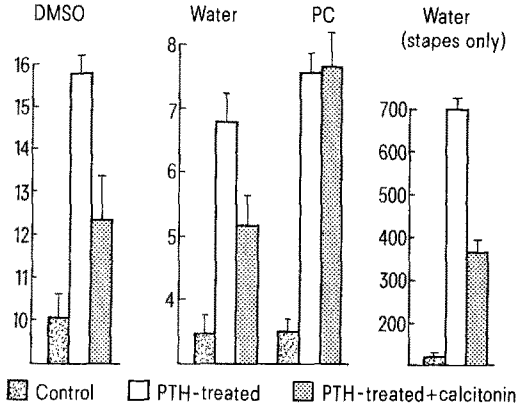
Summary. In view of a recent proposal that calcitonin injections may arrest the bony pathology of otosclerosis, we have tested the possibility of obtaining locally effective concentrations by giving salmon calcitonin in eardrops. Osteolysis of guinea-pig ossicles induced by injecting parathyroid hormone shortly before explantation was markedly inhibited by 3 days prior instillation of the calcitonin in an aqueous vehicle or in dimethyl sulphoxide, but not by a solution in propylene carbonate.

The pathological changes of otosclerosis resemble in some respects the appearance of the earbones in patients where Paget's disease has affected the hearing. Since deafness is sometimes relieved during treatment of the latter by injection of calcitonin[2-5], Diamond[6] has suggested a therapeutic trial of calcitonin in the much commoner condition of otosclerotic deafness. (Otosclerosis is common throughout Europe, the Balkans and the Middle East and among Americans of Caucasian origin, causing clinical deafness in 0.3-0.5% of the adult population[7]).

In view of the disadvantages of treating localized disease of the earbones by systemic hormone injection, we have tested the possibility that effective quantities of calcitonin might be absorbed from suitably formulated eardrops. Guinea-pigs injected with radiocalcium were treated with eardrops of solvent alone or containing calcitonin (250 µg/ml) for 3 days and sacrificed 1 h after i.v. injection of 100 units of bovine PTH (2000 units/mg). Ossicles were removed by opening the temporal bone from its lower surface, remote from the external ear to which the calcitonin had been applied. Ossicles were explanted on to filter discs and cultured in the Fitton Jackson modification of BGJ medium (Gibco-Biocult Ltd), suitably gassed.

Because of the expected low permeability of skin to a peptide and its likely destruction by tissue proteases, we chose the moderately hydrophobic[8] and relatively stable[9] salmon calcitonin, and carried out initial experiments with the aprotic polar solvent dimethyl sulphoxide (DMSO). Other solvents tested were water (containing 0.1% dioctyl sodium sulpho-succinate) and propylene carbonate (PC).



Bar graph illustrating the effect of in vivo parathyroid hormone (PTH) and eardrops with and without calcitonin on the in vitro release of calcium^{45} from guinea-pig ossicles. The ordinates show radioactivity (cpm $\times 10^{-3}$) in 1 ml of bathing medium withdrawn from explants at the end of 20 h incubation in 3 separate experiments (mean + SEM, n = 8).

As shown in the key, the 2nd and 3rd of each set of 3 bars compare the PTH-stimulated release from ossicles of animals which received eardrops of vehicle alone on one side and added calcitonin on the other, while the 1st bar of each set shows the release from ossicles of animals given eardrops of vehicle alone, without injection of PTH. Except when the vehicle was propylene carbonate, the antagonism by calcitonin of the PTH-stimulated resorption was highly significant ($p < 0.01$ in experiments 1 and 2 and $p < 0.005$ in the 3rd experiment, which was carried out with the explanted stapes alone).

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.