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Aflatoxin-Induced Histopathology of Skin in Guinea Pigs

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Effect of oral administration of crude aflatoxins (B_1, B_2, G_1 and G_2) in 40–60 ppb concentration per day to guinea pigs for 20 weeks was studied. Skin showed loss of hairs and development of numerous pettechial haemorrhage sites over it. Carcinoma-like changes were induced in the skin. Formation of "horn pearls" and non-differentiated neoplastic tissue in both the layers of skin was quite frequent. Secondary carcinoma was also observed in the skin where the tumour had plenty of cells, probably of apocrine nature.

Key Words: Aflatoxins, Metastasis, Skin, Guinea pigs, Horn-pearls

Introduction

Aflatoxin-induced histopathological damages in liver and kidney are well known and documented (Newberne et al. 1964 a,b, Butler 1964, IARC 1976, Wyllie & Morehouse 1978). Effect of these mycotoxins on skin are not adequately studied. Lindenfelser et al. (1974) recorded initiation of tumour in the skin of mouse when a high dose of aflatoxin B_1 was administered (25μg).

The normal and expected route of aflatoxin toxicity is through consumption of toxin infested food. In most of the studies conducted so far, the toxins have been administered in experimental animals through injection/intubation in concentrations much higher than the ones, the human populations are usually exposed to. The effect of consumption of crude aflatoxin B_1 in concentrations (40–60 ppb) at which these are normally present in food and feed (Bilgrami 1984), was examined. The details of histopathological changes induced in the skin of guinea pigs are reported here.

Materials and Methods

6–8 weeks old, healthy guinea pigs (*Cavia cavia*) weighing between 140–160 gm respectively were used (stock received from CDRI, Lucknow). All the animals were pathogen free and kept in separate cages at room temperature. One group (6 males) served as control and was fed only with uncontaminated basal diet; while the other group (6 males) was fed with the same basal diet supplemented with

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crude aflatoxins (B_1, B_2, G_1, and G_2) in concentrations ranging from 40 to 60 ppb/day/animal. For obtaining the crude aflatoxin, bakery bread was first infested with a toxigenic strain of *Aspergillus flavus* (BG-185) and then incubated for 12 ± 2 days at $28 \pm 2°C$ for aflatoxin elaboration. Subsequently, the bread was dried and powdered. Toxin-infested bread was orally administered to the treated animals. 3 to 5g of aflatoxin contaminated food (containing 40 to 60 ppb of aflatoxins) was served daily alongwith the normal diets. Diets were properly supplemented with protein, vitamins and minerals.

The animals were sacrificed after 15 to 20 weeks of feeding. Affected tissues were promptly removed and fixed in Bouin's fixative for histopathological studies. Paraffin blocks were cut at 6μ and sections were stained with haemotoxyline-osin.

Results and Discussion

Both dermis and epidermis of the skin of 4 out of 6 guinea pigs were affected. Loss of hairs together with the appearance of red-tinted pin-head sized papillae (sites of pettechial haemorrhage) on posterodorsal surface of the body were quite frequent (figure 1). Biopsy of such skin revealed the presence of thick-walled capsular structures, which were fully keratinized epidermal cells, forming "horn-pearl". These were noted at different stages of development in both epidermis and dermis. When in epidermis (figure 2), the sheath of the pearls were thin-walled, but the epidermis itself being multilayered and thick. When in dermis (figure 3), the horn-pearls were found to be surrounded by a thick sheath made up of small cells arranged in 4 to 5 tiers. The pearl itself appeared to be composed of concentric depositions of hyaline keratine. Some islands of small non-differentiated cells were also encountered within the dermis region (figure 4).

A small nodular structure was observed under the skin in the lumbo-sacral region after 15 weeks of treatment. By the twentieth week, this nodule increased in size, forming a circular tumour of about 1cm diameter. It was attached to the inner surface of the skin, and appeared to be malignant by touch. Histological preparations of such tumour revealed it to be made up of two structural units : a matrix of non-differentiated cell mass, traversed by profuse branching of various tubular structures lined by tall columnar epithelial cells, perhaps of apocrine nature (figure 5).

These studies establish the toxic degeneration and neoplasia of skin in aflatoxin exposed guinea pigs. Non-differentiated neoplastic cell-masses, as found in the dermis portion, show a close similarity to the squamous cell carcinoma in human being. Presence of thick walled capsular structures, which are fully keratinized epidermal cells forming "horn-pearl" is again suggestive of squamous cell carcinoma Grade-I (Lever & Lever 1983). At a still advanced stage, many of the cell-masses and pearls, perhaps, got aggregated together to give rise to a sufficiently large tumour in the lumbo-sacral region. Such tumours comprised of non-differentiated neoplastic cells.

Similar masses of non-differentiated neoplastic cells with hyperchromatic nuclei in the kidney (figure 6) and liver (figure 7) of these animals were found by us, and also reported elsewhere (Ranjan 1985). Whether, the skin itself was the site of primary neoplasia or it was a secondary site of it due to metastasis, is difficult to answer at this stage.

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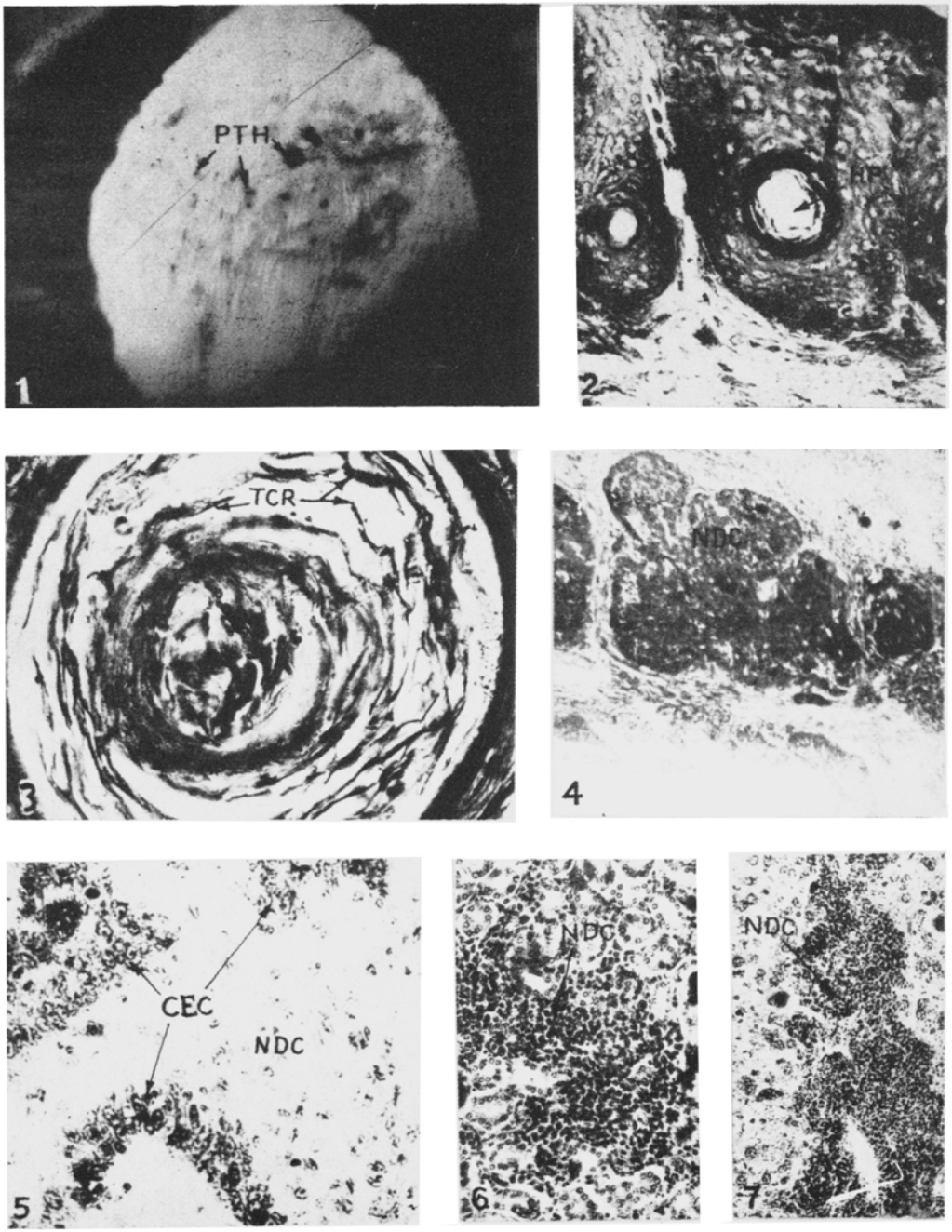

Figures 1–7, 1 Pettechial haemorrhage (PTH) sites on the Postero-dorsal surface of guinea pig; **2,** "Horn-pearls" in the epidermis of the skin (×40); **3,** Horn-pearl in the dermis of the skin showing tiers of concentric rings (TCR) (×100); **4,** Non-differentiated cell masses (NDC) in the dermis of the skin (×40); **5,** Branching of tubular structures with tall columnar epithelial cells (CEC) in the matrix of non-differentiated cell mass (NDC) in the skin (×100); **6,** Non-differentiated cells (NDC) in the medulla of the kidney (×40); **7,** Non-differentiated cells (NDC) in the liver parenchyma (×40)

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