columnar epithelium with apical 'apocrine' blebs. Other ALA show greater epithelial anaplasia which grades into carcinoma-in-situ of the classical ductal type. The essential difference between PL and ALA is that the epithelium of PL is usually normal in appearance; whereas, the epithelium of ALA shows variable anaplasia.

There is, therefore, a striking morphological similarity between the HAN of mammary glands of mice and the PL and ALA of human mammary glands. Our previous data[4] indicate that PL and ALA are more frequent in the mammary glands of patients whose clinical histories suggest that they are at high risk. Further PL and ALA tend to persist after the menopause in a background of atrophic lobules. PL and ALA show a morphological sequence of increasing atypia forming a continuum between normal lobules on the one hand and carcinoma-in-situ on the other[4]. These features of PL and ALA indicate that, like HAN, they may be biologically precancerous.

Some earlier work[6–9] suggests that most mammary dysplasias and carcinomas of humans arize in lobules and their immediate terminal ducts rather than in larger ducts. Our own previous work[10] has clearly shown that the smallest and earliest geographically isolated foci of ductal carcinoma-in-situ are found in lobular structures like PL and ALA.

Recently we have transplanted human PL and ALA into the host glandfree fat pads of nude athymic mice[11]. The results indicate that PL and ALA from breasts over 50 years of age are more likely to show morphological evidence of 'dedifferentiation' than PL and ALA from younger breasts. This suggests that some morphologic instability is present in older persistent mammary lobular lesions, and this instability may be indicative of enhanced precancerous potential.

Our results indicate that the studies of murine mammary tumors are highly relevant to the human case. Inasmuch as human lobules and presumptively preneoplastic lobular lesions such as PL and ALA can now be recognized and removed from living human tissue with a dissecting microscope, new avenues of direct experimentation with excized human mammary tissue become possible.

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The Exotoxin of *Bacillus thuringiensis:* a New C-Mitotic Agent

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Summary. Thuringiensin A, an exotoxin from *Bacillus thuringiensis*, a constituent of the microbial insecticide thuricide has been found to inhibit mitotic spindle, condense and scatter chromosomes. It may therefore be a promizing tool in future cell biological studies.

Although a wide variety of chemicals are known to effect the mitotic process in the root meristems, only a few, like vinblastine, are known to cause C-mitotic effect inhibiting spindle and consequently yielding condensed and well spread chromosome configurations and, on recovery, tetraploid cells[2]. Here we report similar activity by the exotoxin of *Bacillus thuringiensis*, which is a first report of C-mitotic activity by any bacterial toxin.

Effective minimal concentrations of some anti spindle agents on root meristems of *Allium cepa*[2,9]

Compound	Beginning of anti spindle effect (M/ml)	Full anti spindle effect (M/ml)
Exotoxin	3.4×10^{-8} (25 ppm)	1.37×10^{-7} (100 ppm)
Hexanitrodiphenylamine	2.0×10^{-9}	5.0×10^{-9}
Vinblastine	2.2×10^{-8}	4.4×10^{-7}
Vincristine	2.25×10^{-8}	4.4×10^{-7}
Griseo fulvin	2.5×10^{-8}	–
Colchicine	1.25×10^{-7}	2.5×10^{-7}
Phenyl urethane	1.2×10^{-7}	6.0×10^{-7}
p-Hydroxyacetophenone	2.0×10^{-6}	10^{-5}
Antipyrene	4.0×10^{-4}	10^{-3}

The exotoxin, an AMP-derivative[3], is toxic to a wide variety of insect pests[4], plant pathogenic nematodes[5] and also to mammalian systems[6] where it inhibits DNA-dependent RNA polymerase[7]. However, there has been no investigation on the mitotic and chromosomal impact of this toxin.

The toxin is purified from the culture filtrates of *B. thuringiensis* according to the method of Kim and Huang[8]. Growing root meristems of *Allium cepa* are treated in different concentrations (25–1000 ppm) of aqueous exotoxin solutions (Thuringiensin A) for different

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durations (1–12 h) and allowed to recover in water thereafter. Chromosome squashes are prepared following the conventional Feulgen technique.

Among the experiments with different combinations of variables, the 3 h treatment in 100 ppm and upwards resulted in scattered anaphases and in arrested metaphases with well spread chromosome configurations due to spindle inhibition. Although 25 ppm seems to be the minimal effective concentration when used for 6 h, prolonged treatments in other concentrations resulted in chromosome diminution, thus simulating colchicine. Aqueous recovery of the above materials for 24 h accumulated diplochromosomal metaphases and tetraploid cell populations. The minimal effective concentration of the exotoxin compares well with the known spindle poisons[2,9] at the initial stages of C-mitotic activity, and appears to be slightly more efficient towards the full effect (Table). The similarity of exotoxin with vinblastine and vincristine in this connection is noteworthy.

Since the exotoxin is a known RNA polymerase inhibitor[7], the C-mitotic activity has probably resulted from an interference with the synthesis of proteins in the spindle microtubular systems. A few other abnormalities, like phragmoplast inhibition, chromosome bridges, etc, have also been noticed in higher treatments and are under investigation.

The present study suggests the utility of this bacterial exotoxin as a cytological tool, in view of its spindle-inhibiting properties similar to colchicine. For the same reason, nevertheless, there seems to be a need for caution in the extensive use of commercial preparations of *B. thuringiensis* as a microbial insecticide on crop plants.

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Mast-cell Reaction in Precancerous Mouse Skin: an Immunological Response?

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Summary. The promotion phase of carcinogenesis in mouse skin is accompanied by a mast-cell reaction in the upper dermis. Evidence is presented which suggests that this may be an immunological response, whereby distant lymphocytes migrate to the area and, in the presence of young fibroblasts, become transformed into tissue mast cells.

In the course of carcinogenesis in mouse skin a situation develops in the superficial dermis which strongly favours the accumulation of tissue mast cells, the reaction reaching its maximum under a papilloma[1,2]. This curious phenomenon can be induced by promoting agents alone, or, more intensely, by initiation followed by the repeated application of a non-carcinogenic promoter[3,4]. The classical hydrocarbons act in both capacities[5].

The first, and simplest, hypothesis to account for the reaction is to postulate the release of a chemical factor from the overlying, hyperplastic epidermis which acts directly on the dermis[3]. So far, I have failed to identify such a factor. The mast cell in the mouse contains heparin, histamine and 5-hydroxytryptamine, but neither they nor their immediate precursors have proved, on injection, to be capable of eliciting a local development of mast cells. Injections of hyaluronic acid and the chondroitin sulphates, B and C, were likewise ineffective[6].

The absence of mitoses in the new cells, or in the more mature cells already present, suggests a second hypothesis, that the new mast cells stem from distant precursors which only declare themselves when all the conditions for their fulfilment have been met. The first cells, containing a few orthochromatic granules, to appear under the painted epidermis resemble lymphocytes. If this is so, they could either be T-lymphocytes from the thymus, or B-lymphocytes (bursa-equivalent cells) from elsewhere. BURNET[7] has suggested that the mast cell may be an end-cell of the T-lymphocyte. However, mast cells are surprisingly numerous in the untreated skin of so-called 'nude' mice, congenitally lacking a thymus[8]: these cells at least, must stem from non-thymic precursors.

So far as the B-lymphocytes are concerned, CSABA[9] believes that the spleen is the 'mast-cell organ' of the mouse. Yet I have found that splenectomy, prior to painting with DMBA, is without effect upon the emergence of papillomas or upon the mast-cell response[6]. This still leaves a considerable residue of lymphocytes in gut, peritoneum and nodes as possible antecedents for the mast cell, and two lines of evidence suggest that such a transformation may occur.

The first rests on histological findings. Mast cells are rare in encapsulated lymphoid tissue. When, however, non-capsulated lymphoid tissue lies in direct contact with young connective tissue – as in the 'milk spots' of the omentum, the solitary nodules of the gut, or even in lymphoid nodules in the bone marrow – a transformation to mast cells is seen in the boundary zone between the two tissues[2]. Lymphocytes from encapsulated foci must first traverse the lymphatics to reach a suitable site for their full development.

The second line of evidence is immunological. GINSBURG[10,11] has devised an in vitro system in which lymphocytes from a sensitized mouse are cultured on a monolayer of fibroblasts and are there re-introduced to the specific antigen. A massive differentiation into mast cells follows. It will be observed that in both examples,

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