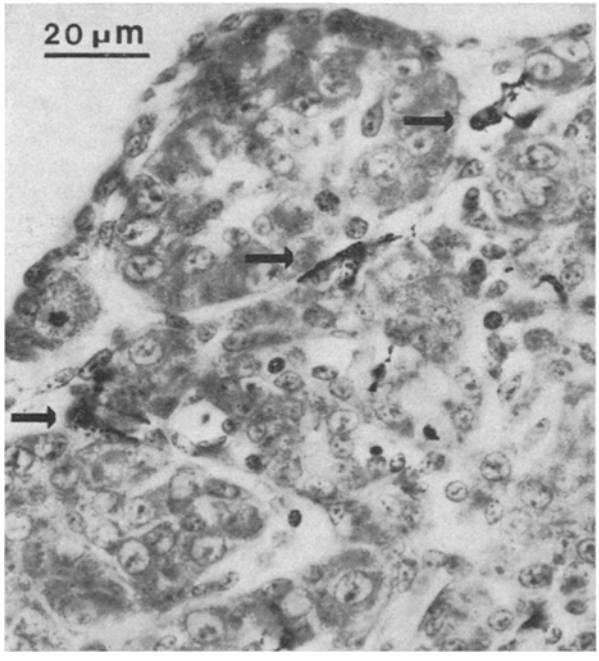

Fig. 3. Section through the rim of the left ovary (from an 8-day-old chicken embryo), grown on CAM for 8 days, in the presence of pigmented peritoneum (from a 10-day-old female quail embryo). A few melanocytes (arrows) have penetrated between the secondary sex cords. Toluidine blue stain.

ovary. We do not know why, during normal development, no melanocytes can be found in the surface epithelium of the Japanese quail gonad, while in the parietal peritoneum, close to the gonadal hilus, numerous pigment cells are present. Our results seem to indicate that the spatial distribution of the melanocytes in the immediate neighbourhood of the gonad determines whether or not penetration will take place.

Avian melanocytes are derived from neural crest material[9]. That melanocytes can settle down in sites of the testis where normally interstitial cells are found, suggests that the latter also may be of neural crest origin. This study demonstrates how quail melanocytes (localized in an easily manipulable sheet of peritoneum) may be used as cellular markers in homospecific as well as in heterospecific tissue associations.

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Persistent and Atypical Lobules in the Human Breast may be Precancerous[1]

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Summary. Persistent human mammary lobules (PL) remaining after the menopause, and certain atypical lobules (ALA) are morphologically similar to the common preneoplastic hyperplastic alveolar nodule (HAN) of mice of strains having a high incidence of mammary carcinoma. This and other evidence suggest that like the HAN of mice human PL and ALA are precancerous.

It is generally agreed that the hyperplastic alveolar nodule (HAN) is the common kind of preneoplastic lesion of the mammary gland of mice having a high incidence of mammary adenocarcinoma[2,3]. HAN are lobuloalveolar in morphology and are more frequently observed in those strains of mice which have a high incidence of mammary adenocarcinoma than in strains which have a low incidence. HAN increase in number with age, and are most frequent in old retired breeders. HAN can be shown by direct experimental means to be preneoplastic in that cancer arizes more frequently in transplanted cell populations derived from HAN than in populations derived from normal mammary cells. Our search for preneoplastic lesions in the human breast provides quantitative morphological evidence that certain kinds of human lobules resembling HAN are precancerous. This evidence is based on quantitative studies of mammary lesions in whole human breasts utilizing a subgross sampling technique with histological confirmation[4].

The purpose of this report is to demonstrate our findings of striking morphological similarities between the common lobular HAN of mice and two kinds of human lobular lesions which we believe to be precancerous to infiltrating duct carcinoma in the human. We have designated these two kinds of human lobules as 1. persistent lobules (PL) and 2. atypical lobules, type A (ALA), and will define these further in the text.

Mammary glands were obtained from 24 female mice as follows: C3H (retired breeders), 2 mice; BALB/c (retired breeders), 10 mice; CF2 (retired breeders), 3 mice; GR (old virgins), 9 mice. Whole glands were fixed overnight in 10% buffered formalin, stained with iron hematoxylin at pH 1.3–1.5, dehydrated, and stored in methylsalicylate[5].

210 whole human mammary glands were fixed for 4 weeks in 10% buffered formalin, embedded in 10%

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gelatin, sliced at 2 mm, stained with hematoxylin, dehydrated, rinsed in acetone, and transferred to methysalicylate in sealed plastic bags. The procedure is described in detail elsewhere[4].

Both mouse and human mammary glands were then examined for focal lesions with a dissecting microscope and subgross (2.5–10 ×) photographs of all lesions made. Each lesion was then dissected out of the whole mammary gland or slice and embedded in paraffin. A conventional histology slide stained with hematoxylin and eosin was prepared for correlation with the subgross appearance.

The subgross and histologic appearance of HAN was similar in all strains studied and typified by Figures 1–3. The HAN is lobular and exists in a glandular background in which normal lobules are either very small or totally lacking. Individual alveoli are lined by an inner layer of secretory epithelial cells and an outer layer of myoepithelial cells. Secretory material may be present in the lumens and cells. A few HAN (about 5%) show variable epithelial anaplasia.

The persistent lobule (PL) of humans is a morphologically similar lobule existing in a postmenopausal glandular background composed of small atrophic lobules (Figures 4–6). Individual terminating ductules are usually lined by epithelium similar to that observed in the HAN, and identical to that observed in normal human lobules.

The structure we have previously designated atypical lobule, type A (ALA) is, like HAN and PL, a lobule consisting of a grouping of blindly ending ductules (Figures 7–9). The ductules in the example are lined by tall

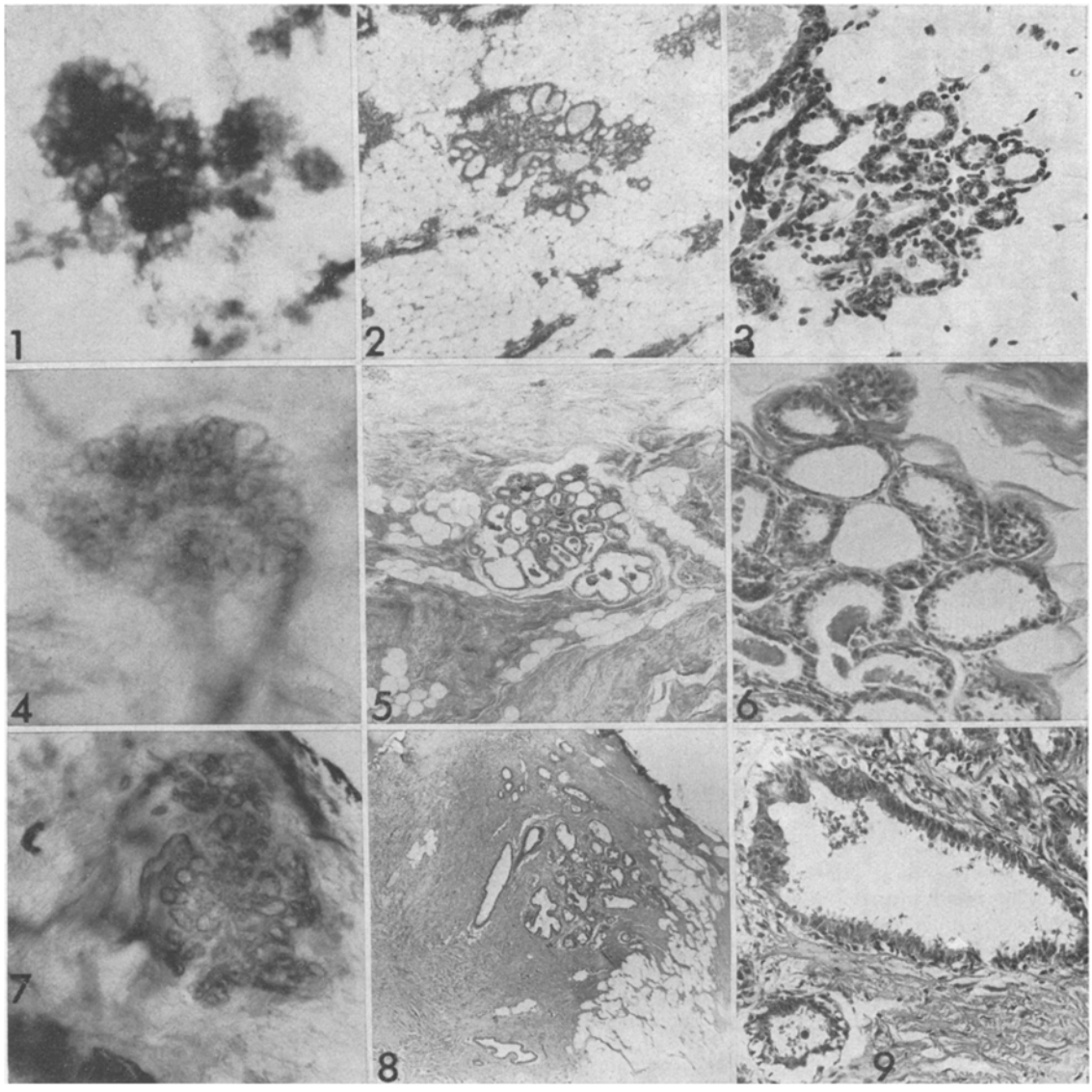

Fig. 1. Hyperplastic alveolar nodules (HAN) of mouse of GR strain. Note individual terminal ductules (or alveoli). ×40.
Fig. 2. Histology of the same HAN shown in Figure 1. ×46.
Fig. 3. Same HAN shown in Figures 1 and 2. Terminal ductules have small amount of secretion. ×205.
Fig. 4. Persistent lobule (PL) of post-menopausal human female. Individual ductules are clearly seen. ×30.
Fig. 5. Histology of the same PL shown in Figure 4. ×29.
Fig. 6. Same PL shown in Figures 4 and 5. ×143.
Fig. 7. Atypical lobule, type A (ALA) of post-menopausal human female. Note individual ductules. ×14.
Fig. 8. Histology of the same ALA shown in Figure 7. ×16.
Fig. 9. Same ALA shown in Figures 7 and 8. Note individual ductules. ×158.

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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