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## Newly synthesized elastin is associated with neoplastic epithelial cells in human mammary carcinoma[1]

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*Summary.* Indirect immunofluorescence with a purified antiserum to human foetal elastin has identified newly synthesized elastin on the membranes of neoplastic epithelial cells in human mammary carcinoma.

The focal accumulation of elastic tissue (elastosis) is a recognized histological feature of scirrhous mammary carcinoma in women[3,4]. Studies utilising elastase digestion[3] and electron microscopy[5-7] have confirmed the presence of elastin and elastic tissue microfibrils in this material. The ultrastructure of elastic fibres within the tumour mass differs from that of mature arterial elastica, the tumour fibres being thinner, randomly orientated and containing a lower ratio of amorphous elastin to microfibrils[6,7], all of which are characteristic features of newly synthesized elastic tissue[8]. The phenomenon of tumour elastosis has usually been regarded as a stromal reaction to the presence of infiltrating malignant epithelium[5,7]. However, it has also been suggested that elastic tissue may originate from the tumour cells themselves[6]. This view is supported by the results of the present study in which synthesized elastin in tumour tissues has been demonstrated by immunofluorescence to be intimately associated with neoplastic epithelial cells.

Insoluble elastin was purified from breast carcinoma tissue and from adult and foetal human aortas after decalcification in 0.5 M EDTA, 0.01 M Tris HCl, pH 7.4[9], autoclaving with distilled water and repeated extraction with 0.1 N NaOH at 98 °C[10]. Amino acid analyses of these preparations are shown in the table. Despite exhaustive extraction, the tumour elastin remained contaminated with small amounts of glycoprotein (about 5%). This is reflected in larger amounts of aspartate in the tumour elastin than in the pure feotal aortic elastin. The amounts of the cross linking amino acids desmosine and isodesmosine in relation to the amount of lysine give some indication of the age of the elastin. Tumour elastin is poorly cross linked (desmosines/lysine ratio = 0.35) and immature in comparison to adult aortic elastin (desmosines/lysine ratio = 0.55). Similar immaturity is observed in foetal aortic elastin (desmosines/lysine ratio = 0.25).

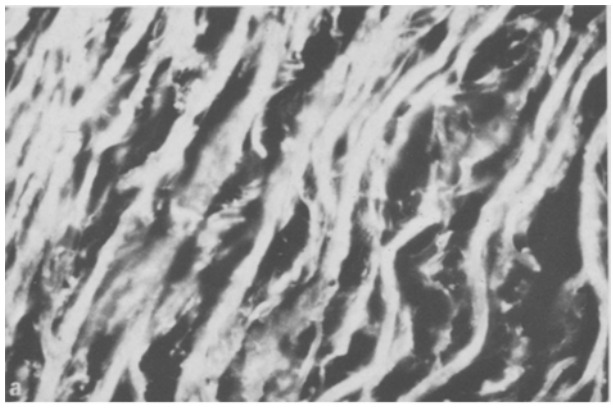
Using the highly purified foetal aortic elastin as an antigen, antisera were raised in sheep to both the insoluble protein and its oxalic acid solubilized peptides ($\alpha$-elastin)[11]. The methods used and characteristics of the antisera produced have recently been described[12]. Purified antibodies to human foetal $\alpha$-elastin did not cross-react with elastic fibre glycoproteins or purified type I and III collagens but gave strong reactions in passive haemagglutination tests with human $\alpha$-elastins. The maximum reciprocal titre achieved in this system when foetal $\alpha$-elastin was used as the coating antigen was 4 times that achieved with adult $\alpha$-elastin, indicating preferential affinity of the antibodies for immature elastin[12].

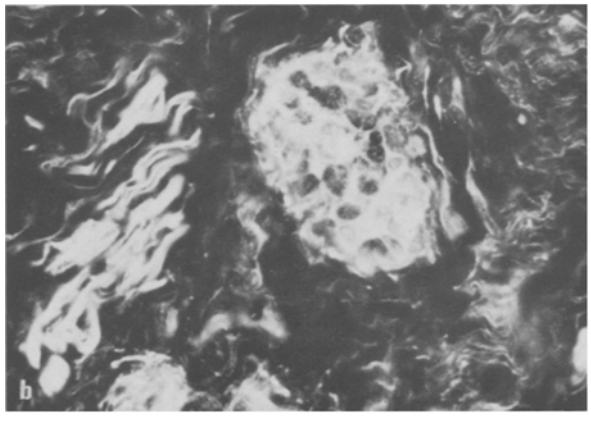
Elastin was localized by indirect immunofluorescence in fresh frozen sections of surgically excised human tissues. Binding of the antibody in sections from child aorta resulted in bright specific fluorescence over large and small elastic lamellae (figure, a). In mammary carcinomas with

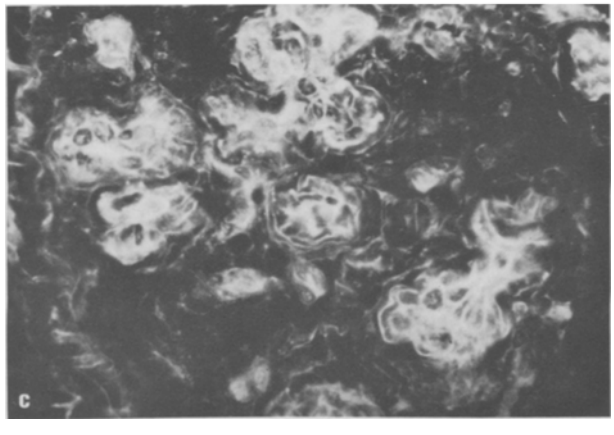
Amino acid composition of elastins extracted from human mammary carcinoma and adult and foetal human aorta (values are given as moles/1000 moles)

| Amino acid | Human mammary carcinoma elastin* | Human adult aortic elastin | Human foetal aortic elastin |
|---|---|---|---|
| Hydroxyproline | 16.1 | 18.2 | 12.6 |
| Aspartic acid | 11.2 | 11.5 | 3.8 |
| Threonine | 13.5 | 13.7 | 11.5 |
| Serine | 10.9 | 9.3 | 8.5 |
| Glutamic acid | 27.5 | 28.2 | 18.8 |
| Proline | 144.5 | 154.5 | 122.0 |
| Glycine | 263.3 | 252.1 | 288.8 |
| Alanine | 211.5 | 207.5 | 217.1 |
| Valine | 130.2 | 130.0 | 140.1 |
| Methionine | 3.3 | 5.3 | 2.5 |
| Isoleucine | 26.9 | 29.2 | 27.3 |
| Leucine | 65.2 | 68.6 | 65.1 |
| Tyrosine | 22.5 | 21.2 | 21.5 |
| Phenylalanine | 25.1 | 24.4 | 26.0 |
| Lysine | 12.2 | 9.1 | 10.7 |
| Histidine | 1.7 | 0.8 | 0.6 |
| Arginine | 8.6 | 9.7 | 7.1 |
| Desmosine | 2.0 | 2.8 | 1.4 |
| Isodesmosine | 1.9 | 2.2 | 1.3 |

* Mean of analyses from 4 elastotic tumours.







Fresh frozen sections (6–8 μm) were incubated with purified antisera to human foetal aortic *a*-elastin for 60 min, washed in saline for 90 min and reacted with fluorescein isothiocyanate-conjugated anti-sheep IgG for 30 min. Following a further 120-min wash in saline, the sections were mounted in von Apathy's medium and photographed under oil immersion and epifluorescence using a Zeiss ultraphot 3B photomicroscope fitted with a IIIRs incident fluorescence illuminating attachment. *a* Human child aorta. Large and small medial elastic fibres are visualized. ×375. *b* Human mammary carcinoma. Stromal elastic fibres and a nest of tumour cells both show positive immunofluorescence. ×375. *c* Human mammary carcinoma. Tumour cell clumps bind specific antibody strongly. ×375.

elastosis, newly synthesized elastin was always identified around ducts. The 7 tumours examined were all infiltrating ductal carcinomas and in 3 of these, elastin was located in close association with the plasma membrane of the tumour cells themselves (figure, b). In this type of mammary carcinoma, malignant epithelial cells occur singly or in small nests of cells in the fibrous stroma. In immunofluorescence studies, the binding of anti-elastin antibodies clearly silhouetted these tumour cell nests and identified elastin antigen closely adjacent to the cell surfaces (figure, b and c). The binding of antibody at this site was not a non-specific event, since in non-elastotic areas, carcinoma cells were completely unstained by this technique.

The localisation of elastin on neoplastic epithelial cell surfaces in scirrhous carcinoma strongly suggests that these cells are responsible for its synthesis. It is conceivable that hydrolyzed elastin fragments produced by the action of tumour elastases might be absorbed on to the surface of the cells or even internalized by endocytosis. However, electron micrographs (not shown) of tumour cells in elastotic sites revealed few surface endocytotic vesicles and we consider surface absorption unlikely. Rather, our experiments support the ultrastructural evidence of Douglas and Shivas[6] in favour of the theory that tumour cells actively secrete elastin. The recognition of elastin secretion by neoplastic epithelium does not exclude the possibility that stromal fibroblasts may also contribute to elastosis. However, we did not observe binding of anti-elastin antibodies to stromal fibroblasts.

The malignant cells of human scirrhous mammary carcinoma are characteristically surrounded by a dense basement membrane, lack surface microvilli and frequently contain numerous intracytoplasmic myofibrils[13]. These ultrastructural features are also characteristic of aortic smooth muscle cells which are known to synthesize elastin[14,15]. This study suggests that elastogenesis may be a feature of cells with myofibrillar differentiation and that the capacity to synthesize elastic tissue is maintained in mammary epithelial cells and expressed following malignant transformation in scirrhous carcinoma.

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