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# Three dimensional anatomy of complete duct systems in human breast: pathological and developmental implications

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## Abstract

**Aims**—To reconstruct the arrangement in space of all major ducts and their branches from nipple to periphery of a human breast obtained at necropsy.

**Methods**—Duct tracing through cleared haematoxylin stained 2 mm sub-gross coronal slices of a complete necropsy breast and computer modelling of duct territories.

**Results**—All branches were traced for 10 complete duct systems of a single breast from a 19 year old girl. Their complexity prevented comprehensive modelling of individual ducts and rami using available computer software, but the territories (catchments) drained by individual duct systems did not overlap and could be reconstructed. Catchment volume and length of the central unbranched duct draining each catchment varied greatly. Duct spacing showed non-random uniformity which is also seen in rodent mammary glands.

**Conclusions**—These spatial relations are consistent with mutual growth inhibition between duct systems during mammary development. Although there is no clear morphological distinction between mammary duct end buds and lateral buds in women, the present study does suggest that processes of branching morphogenesis occurring during development of the breasts in women do show some analogies with the growth of end buds/lateral branches/alveoli during rodent mammary gland development. Rodent models of mammary development may usefully suggest hypotheses about human breast biology. Less laborious methods of three dimensional reconstruction of mammary ducts and their branches from sub-gross slices, allowing more specimens to be studied, would be valuable for the study of normal human breast development and mammary intraepithelial neoplasia. Increasing power and decreasing costs of high definition image processing hardware and software may make such endeavours practicable.

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## Introduction

### ANATOMY OF HUMAN BREAST PARENCHYMA

Descriptions of the major ducts and how their branches are arranged in the human female breast are few and contradictory. According to Cheate and Cutler,<sup>1</sup> 'Milk is conducted to the nipple by 15 to 20 main ducts, the peripheral branches of which overlap each other' (p 4) and '... pathological changes ... do not demonstrate a division of the breast into lobes' (*ibid*). Page and Anderson<sup>2</sup> say 'The breast's system of branching ducts is arranged in a segmental, roughly radial pattern ... This arrangement divides the breast into poorly defined segments or lobes but it is stressed that these may overlap and have no macroscopic or anatomical delineation (p 6)'. According to Tavassoli,<sup>3</sup> the breast has 15 to 20 segments or lobes, but 'the lobes are ill-defined and not appreciated on gross inspection'. Haagensen<sup>4</sup> baldly asserts the existence of '20 or more lobes' (p 8). Sloane<sup>5</sup> describes 'a number of separate glandular trees or segments' (p 4), thereby seeming to imply discrete lobes. McCarty and Tucker<sup>6</sup> refer to 15 to 25 lobes, and state that 'Each lobe is surrounded by connective tissue', a view discordant with everyday pathological experience. Neither Dawson<sup>7</sup> nor Richardson<sup>8</sup>, in otherwise comprehensive reviews, shed much light on the subject.

### STUDY BACKGROUND

The normal parenchymal anatomy of the human female breast is important. The distribution of ducts and their branches in space records the trajectories and branchings of ducts during breast growth, which may yield insights into control of breast development. These processes may, in turn, influence pathological events in the breast. Experimental studies in rodents are yielding new information about mechanisms of mammary development<sup>9-11</sup> and morphological considerations are central to these studies.<sup>12</sup> Detailed examination of human breast parenchymal anatomy may suggest whether mechanisms defined in experimental rodent models are likely to apply to human breast development, which, despite similarities, exhibits noticeable differences. Extensive development of acinar structures (terminal duct lobular units) in human breast before pregnancy contrasts with rodent mammary development, and specifically human epithelial-stromal interactions are implied by the

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Figure 1  
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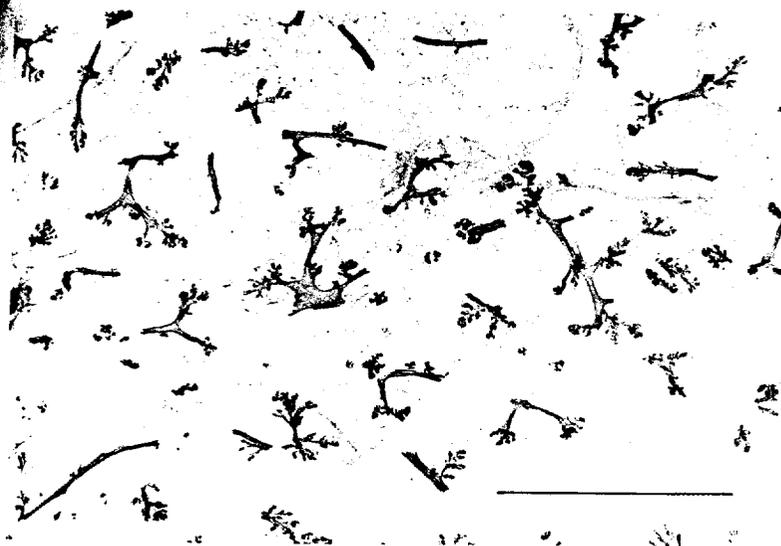


Figure 1 Section of human breast tissue, 2 mm thick, stained with haematoxylin and cleared with methyl salicylate. Many ducts traverse the slice, branch, and give rise to terminal duct lobular units. Scale bar is 10 mm.

increase in stromal volume during development responsible for the characteristic form of the adult human female breast, which is not seen in a species as closely related to us as the gorilla.

Duct anatomy is also relevant to the development of mammary intraepithelial neoplasia, including ductal and lobular carcinoma in situ (DCIS and LCIS) and their putative precursors. By definition, clonal expansion of such lesions must be confined to the branches of a single duct system. Extensive DCIS within a mastectomy is not uncommon in the practice of surgical pathology. Are these lesions present within a single duct system, or more than one? Details of duct anatomy bear directly on this question, which is important for the better understanding of mammary carcinogenesis.<sup>13</sup>

The purpose of the present study was to investigate duct distribution in normal necropsy breasts of pre-menopausal women, and to develop techniques which might help to elucidate physiological and pathological states in human breast, as the informative whole-mount technique<sup>8,9,14</sup> used in the study of rodent mammary development has done.

## Methods

### SUB-GROSS TISSUE SLICES

Sub-gross sections were prepared using techniques<sup>15</sup> communicated by Dr Hanne Jensen (University of California). Breast tissue was collected from five hospital necropsies of pre-menopausal women with informed consent to remove tissue for research. Breasts were removed subcutaneously with the nipple and areola; normal external contour of the body was restored. Intact breasts were packed in cotton wool soaked in 10% neutral buffered formalin and fixed for a minimum of several weeks.

Fixed breasts were washed overnight in running water, embedded in 2% gelatin and frozen overnight at  $-20^{\circ}\text{C}$ . Embedded breast was released from the mould with warm water, refrozen, and mounted in a butcher's slicing machine for complete conversion into 2 mm

slices in the coronal plane. Adherent gelatin was removed with warm water and slices were washed in cold running water for 30 minutes. Slices stained with constant agitation in freshly filtered Harris's haematoxylin (three minutes) were decolourised in acid water (980 ml distilled water, 20 ml concentrated hydrochloric acid) for five minutes, rinsed in water, treated with 5% ammonia water for five minutes, and washed overnight in running water. Harris's haematoxylin was prepared by dissolving 5 g haematoxylin in 75 ml ethanol and 100 g ammonium alum in 1 litre of distilled water. The two solutions were mixed and 500 mg sodium iodate added with stirring 10 minutes before use.

Parenchyma should be deeply stained with minimal staining of connective tissue. Excessive background was reduced by destaining in acid water. Slices were dehydrated through 95% ethanol (three changes, eight to 24 hours each), 99% ethanol (same schedule), cleared in methyl salicylate (eight to 24 hours), and sealed without bubbles in Kapak bags with fresh methyl salicylate.

### THREE DIMENSIONAL RECONSTRUCTION

Photographic prints three times life size were prepared from tissue slices. From these, ducts and lobules were traced onto acetate sheets, from the nipple to the periphery of the breast. All branches derived from single central ducts were traced to the deep aspect of the breast and towards the periphery and colour coded to indicate which duct system they belonged to. Constant stereomicroscopy of individual slices was needed to verify connections from one slice to the next. Three dimensional reconstruction was performed using a VIDS-V digitising tablet and software (Analytical Instruments, Pampisford, Cambridge, UK) and VIDS-3D reconstruction software on an IBM PS/2 microcomputer. As the software package was not able to handle complete reconstruction of all the branches of the parenchymal tree, reconstruction was confined to duct territories (catchments). Individual duct systems are displayed as sections through the envelope which includes all branches of that system in each successive slice; this envelope was fitted by eye and digitised. The digital model was stretched fourfold in the z-axis for clarity.

## Results

Satisfactory staining of parenchyma was achieved in the tissue slices. Figure 1 shows a slice at low power; fig 2 details a duct approximately 0.5 mm in diameter traversing a tissue slice. Six separate exit and entry sites are arrowed, at which the ducts enter adjacent sections on either side of the slice. To trace ducts satisfactorily, it was necessary to determine connections of all of these branches, and their subsequent ramifications, by stereomicroscopy of original tissue slices. It was not possible to determine from photographs in which plane ducts were leaving the tissue slice. This time consuming process did not permit

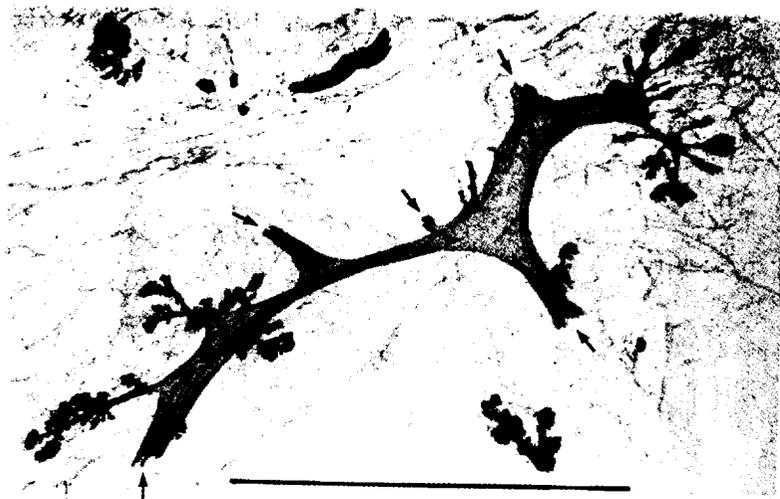


Figure 2 Section of breast tissue, 2 mm thick, stained with haematoxylin and cleared in methyl salicylate. Branches from this segment of a duct system leave the section in six different places (small arrows). It is not possible to tell from the two dimensional photograph which plane (superficial or deep) they then enter. Scale bar is 5 mm.

reconstruction of parenchymal trees from more than one breast from a mentally retarded 19 year old girl who died of a respiratory infection associated with untreated primary lymphoblastic lymphoma of the ovary. No stigmata of any known syndrome were present.

Ten ducts were completely traced, representing about half of the duct systems present within a single breast, including all those present in the central part of the breast. Inspection of acetate tracings showed variation in the tissue volume and distribution of ducts and their branches. These differences are best appreciated from the three dimensional reconstructions (fig 3), which confirm the existence of distinct, non-interpenetrating catchment volumes derived from single central ducts in human breast. For clarity, the model is depicted at four stages of complexity, with two, six, eight, and 10 duct systems included (figs 3A-3D).

#### *Variation in catchment volume and length of unbranched duct*

Figure 3A shows the smallest catchment (green) and one of the larger ones (blue I). There is a 20 to 30-fold difference in tissue volume between these two catchments. Another difference is that the unbranched, central duct of blue I is short (4 mm) but the green duct passes back from the nipple for about 28 mm before ramifying in the deep part of the breast, just anterior to the pectoral fascia. Similar differences can be observed between other duct systems—for example, the small, deeply placed catchment (grey I) in fig 3B and the large, early branching catchment (orange) in fig 3C.

#### *Variations in catchment form*

There are variations in catchment boundary form. Some catchments have predominantly convex profiles in section (fig 3A, catchment blue I; fig 3D, catchment grey II in foreground),

while others are concavo-convex in section (catchment orange), flattened (deep extension of catchment red I) or biconcave (catchment turquoise). Some have smooth profiles, while others appear scalloped or irregular (catchment magenta, catchment red II). Straight catchment edges are due to the edges of the acetate sheets used for duct tracing, beyond which ducts were not traced further.

#### **Discussion**

The mammary parenchymal trees, while modified by subsequent events, preserve an archaeology of breast development. The present study, although of a single breast, permits some hypotheses about human breast development, in light of what is known about mammary development in animals.

Mammary development begins in conjunction with complex hormonal changes associated with intrauterine life, but most development occurs in the postnatal period, in response to the changing hormonal environments associated with pituitary and ovarian function, mediated through and modified by complex, often embryo-like<sup>10</sup> stromal-epithelial interactions.<sup>17</sup> Studies are now describing some of the complexities of this process, including activation of *Wnt*<sup>10,18,19</sup> and *Hox*<sup>20</sup> genes and expression of growth factors like transforming growth factor (TGF) and epidermal growth factor.<sup>21</sup> It is not appropriate to review this complex subject in detail here, but local inhibitory factors have a profound influence on branching morphogenesis in the developing mammary gland, as demonstrated by Faulkin and DeOme in classic experiments,<sup>14</sup> and more recently similar effects on the growing end buds of rodent mammary glands have been demonstrated with the three mammalian isoforms of TGF.<sup>9,22,23</sup> That analogous inhibitory mechanisms operate during human breast development is suggested by the regularity of duct spacing in human breast (well seen in fig 1) and by the success with which different duct systems seem to exclude potential competitors from the tissue volume which they have successfully colonised, effectively permitting no interpenetration between independent systems.

In rodent mammae, three types of budding growth occur.<sup>22</sup> End buds effect duct elongation, lateral buds form ducts over shorter distances, and alveolar buds establish the secretory parenchyma, usually when pregnancy is established. Although well developed end buds like those of rodent mammary glands<sup>12</sup> are not defined in the developing human breast, the lobar anatomy we describe suggests that analogous, distinct tiers of morphogenetic processes may operate. The lack of interpenetration by competing duct systems implies that mutual inhibition of lateral branches is highly effective. If, however, this inhibition was effected by a single mechanism operating at all stages of branching morphogenesis, then any duct system failing to colonise a volume of potential breast tissue by lateral branching should suffer complete inhibition. This is not the case. In

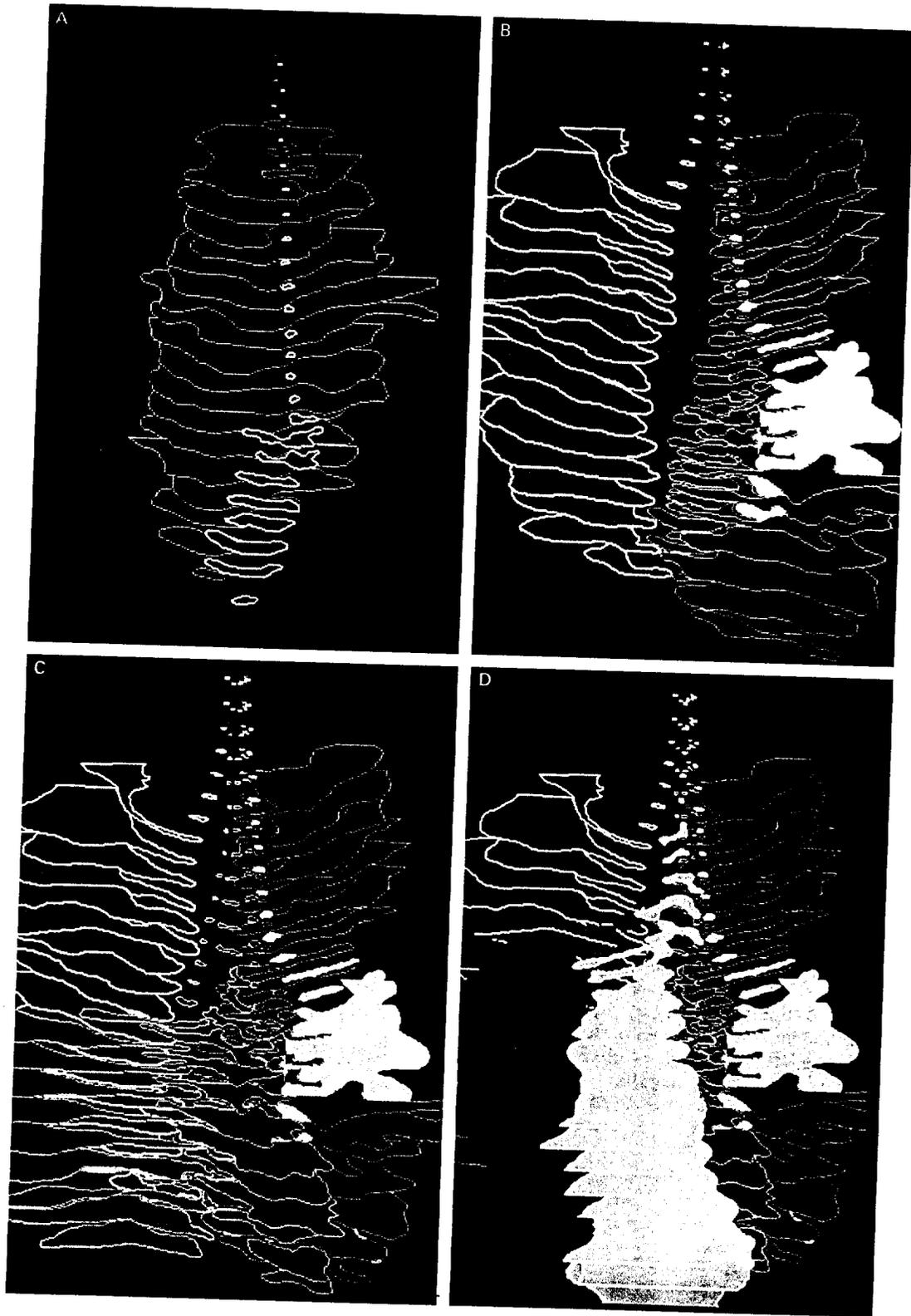


Figure 3 (A) Three dimensional reconstruction of two breast duct territories (breast catchments): blue I and green. (B) Three dimensional reconstruction of six breast duct territories (breast catchments): as fig 3A, plus red I and red II (superficial and deep), orange, and grey I. (C) Three dimensional reconstruction of eight breast duct territories (breast catchments): as fig 3B, plus magenta and turquoise. (D) Three dimensional reconstruction of 10 breast duct territories (breast catchments): as fig 3C plus blue II and grey II.

the breast we studied, at least five catchments show no branching of their major duct until a considerable depth within the breast. Thus, duct elongation can occur in circumstances effective in inhibiting branching morphogenesis, which suggests the existence within developing human breast of an end bud ana-

logue which is at least partly refractory to growth inhibition by competing duct systems. That none of the five long unbranched ducts penetrated another catchment territory implies some responsiveness to inhibitory stimuli.

It seems that morphological analyses may still illuminate growth and development, and

suggest that processes analogous to those in developing rodent mammary glands may occur during breast growth in women. This is of interest given the advances made recently in that field.

Our findings are provisional and should not be over-interpreted. We have only studied one breast in detail, and it would be imprudent to assume that duct growth occurs identically in all breasts. While ductal and lobular carcinoma in situ may occupy circumscribed volumes of breast tissue, reminiscent of the discrete catchment volumes we describe, this pattern is not always observed, and different patterns of breast duct development may occur in different women. It would clearly be desirable to study more duct systems, but their complexity makes wide application of manual methods impractical. The falling cost of computers capable of rapidly processing complex image files may make such studies feasible, and they merit exploration, but will probably require the development of specialised software for the task.

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