

# Anatomy of the Nipple and Breast Ducts Revisited

Susan M. Love, M.D., M.B.A.<sup>1,2</sup>  
Sanford H. Barsky, M.D.<sup>3</sup>

<sup>1</sup> Department of General Surgery, David Geffen School of Medicine, University of California–Los Angeles, Los Angeles, California.

<sup>2</sup> Dr. Susan Love Research Foundation, Los Angeles, California.

<sup>3</sup> Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California–Los Angeles, Los Angeles, California.

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Dr. Love is the founder of ProDuct Health, Inc., which commercialized the intraductal catheter. ProDuct Health has since been acquired by Cytoc Corporation, and Dr. Love currently holds stock in and serves as a consultant to Cytoc.

Address for reprints: Susan M. Love, M.D., M.B.A., P.O. Box 846, Pacific Palisades, CA 90272; Fax: (310) 230-1612; E-mail: slove@earthlink.net

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**BACKGROUND.** Increasing interest in the intraductal approach to the breast has necessitated revisiting the anatomy of the breast.

**METHODS.** Using six different complementary in vivo and in vitro approaches, the authors determined the number, distribution, and anatomic properties of the ductal systems of the breast, which extend from the nipple orifices to the terminal duct lobular units.

**RESULTS.** More than 90% of all nipples examined contained 5–9 ductal orifices, generally arranged as a central group and a peripheral group. Each nipple orifice communicated with a separate, nonanastomosing ductal system, which extended to the terminal duct lobular unit.

**CONCLUSIONS.** Increased knowledge of the ductal anatomy of the breast and the ability to access the nipple ductal orifices will provide a foundation for the intraductal approach to the breast. *Cancer* 2004;101:1947-57.

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Recent attempts to access the ductal systems of the breast by endoscopy<sup>1,2</sup> or lavage<sup>3</sup> have made it imperative to obtain a more accurate description of the ductal anatomy than presently exists. One of the key issues being evaluated currently in ductal lavage is how many ducts need to be lavaged for effective screening. One school of thought argues that the duct that is secreting most actively and the duct that, thus, can be accessed most easily is the *sentinel* duct, which reflects, through either cytology or some other as yet undefined intermediate or surrogate endpoint marker, the status of the breast. Another school of thought argues that this just is not true and that all ducts, or at least a greater number of ducts, should be sampled. If the latter argument holds true, then a knowledge of an individual's specific ductal anatomy would be very important.

Since the studies of Wellings,<sup>4</sup> we have known that breast carcinoma is primarily a disease of the terminal duct lobular units. Detailed histologic descriptions of the terminal duct lobular units and electron photomicrographs of epithelial microvilli obscure the fact that we do not know how they connect to the nipple orifices. Cooper, in one of the earliest studies on record (in 1845),<sup>5</sup> described over 200 dissections of breasts in which he reported not only on the anatomy but also on the development and involution of the breasts of women, men, and assorted animal species. Not until 1972 was there any attempt to study the ductal anatomy of the breast systematically with modern techniques. In that attempt, Sartorius and Smith<sup>6</sup> obtained > 2000 ductograms and initiated an analysis of the ductal anatomy. Teboul and Halliwell published their elegant atlas of ultrasound and ductal echography of the breast in 1995.<sup>7</sup> They described > 6000 ultrasound studies of the breast ducts and lobules subtitled their book, *The introduction of anatomic intelligence into*

*breast imaging.* More recently, Ohtake et al.<sup>8</sup> ran computer simulations based on surgical quadrantectomies to analyze ductal anatomy, and Moffat and Going reported a three-dimensional computer model based on submacroscopic coronal slices of an autopsy breast.<sup>9</sup> Those independent studies of the ductal anatomy were contradictory in the number of ductal orifices, the number of different ductal systems, and the presence of anastomoses among different ductal systems. In an attempt to resolve these controversies and to facilitate new screening and chemopreventive approaches, we determined the number, distribution, and anatomic properties of the ductal systems using six different (although complementary) approaches, all of which were based on the successful identification of and gaining of access to the nipple ductal orifices.

## MATERIALS AND METHODS

All studies were approved by the University of California–Los Angeles Human Subjects Protection Committee and Institutional Review Board. Informed consent was obtained from participants.

### Approach 1: Direct Observations of the Number and Distribution of Nipple Ductal Orifices by In Vitro Serial Sectioning and Three-Dimensional Digital-Image Analysis

To understand the milk duct orifices better, we performed an extensive evaluation of 10 nipples that had been fixed in formalin and paraffin, embedded, and subjected to coronal sectioning. Fifteen-micron (15  $\mu$ ) step sections were taken through each nipple (an average of 1000 sections per nipple). Sections were stained with hematoxylin and eosin and, when appropriate, were combined with anticytokeratin immunocytochemistry to detect the ductal orifices and to distinguish them from sebaceous and sweat ducts and isolated keratin plugs. We used a pancytokeratin antibody called AE3 (DAKO Laboratories, Glostrup, Denmark). This is a broad-spectrum cytokeratin antibody that recognizes the family of Type II keratins, which include keratins with molecular weights of 65–67 kilodaltons (kD), 64 kD, 59 kD, 58 kD, 56 kD, and 52 kD. The cytokeratin immunocytochemistry was used together with the hematoxylin and eosin staining to distinguish the breast ducts from sebaceous glands, sweat ducts, and keratin plugs. Both the breast ducts and the sweat ducts stained with cytokeratin, whereas the sebaceous glands and the isolated keratin plugs did not. The breast ducts coursed deep into the breast, whereas the sweat ducts remained close to the surface of the nipple and, thus, could be separated. The two-dimensional sections that indicated the presence of ductal orifices were subjected to additional, three-dimensional digital-image analysis, which utilized a digital-imaging system that included a Dialux

microscope (Leitz, Heerbrugg, Switzerland) linked to a Vidicon camera, a personal computer (IBM, White Plains, NY) with a PCVision digitizer (Imaging Technology, Bedford, MA), and Microscience software (Microsciences, Bristol, PA).

### Approach 2: Direct In Vivo Observations of Ductal Orifices in Lactating and Nonlactating Women

To determine the number and pattern of ductal orifices, we decided initially to study lactating women. This would insure that the orifices we identified were indeed ductal orifices and not sebaceous glands or sweat ducts, as described originally by Cooper and by Sartorius and Smith.<sup>5,6</sup> With the assistance of La Leche League (a national breast-feeding support group) and a local breast-feeding support network located at the Pump Station (Santa Monica, CA), a commercial source of breast pumps, we approached the lactating women. Two hundred nineteen lactating women were enrolled in this study. After obtaining informed consent, women were asked to empty their breasts to reduce pooling of milk on the nipple. A trained observer determined the number and location of milk-producing nipple ductal orifices before pooling occurred. This approach tended to underestimate the number of orifices, because it was more difficult to count many orifices than a few. The same observer studied all of the women in the study in an effort to minimize interobserver bias, although the possibility of a systemic bias could not be ruled out. Immediately after visualizing the lactation, the observer diagrammed the orifices on a prepared grid. The location of the orifices was characterized relationally, because the position of the women and their breasts were not standardized perfectly. Nonetheless, the resulting observations estimates the number of nipple duct orifices and their approximate location. We studied 219 lactating women. We performed a *K*-means cluster analysis<sup>10</sup> to determine empirically the number of nipple orifices and their pattern.

### Approach 3: Direct Observations of the Number and Distribution of Nipple Ductal Orifices by In Vitro and In Vivo Transareolar Dye Injection

In an attempt to confirm our in vivo and in vitro findings, we studied 13 detached breasts and 1 in vivo specimen. In mastectomy specimens (13 breasts) and in 1 patient, a transareolar dye injection technique was employed to identify the ducts. This technique made use of the fact that the lactiferous sinuses taper as they course to the surface of the nipple, therefore causing a natural wicking of dye up the duct to the nipple. Water-soluble lymphazurin (1–2 cm<sup>3</sup>) was introduced transareolarly into the base of the nipple

with a syringe. The dye diffused into lactiferous sinuses and wicked up through capillary action, causing dark-blue spots to appear on the nipple surface. The number and location of the orifices was recorded. This procedure was repeated in a woman using lymphazurin mixed with lidocaine and was tolerated without difficulty. The nipple ductal orifices were identified as in the in vitro studies.

**Approach 4: Direct Observations of the Number, Distribution, and Physical Properties of the Underlying Ductal Systems of the Breast by In Vitro Ductal Orifice Cannulation, Dye Instillation, and Histologic Sectioning**

After identifying the nipple ductal orifices with the transareolar dye injection, as described above for Approach 3, the orifices were cannulated and dilated with guide wires prior to the placement of a single-lumen or double-lumen catheter. In each nipple ductal orifice that was catheterized in this manner, a water-insoluble dye of a different color was instilled, and the breasts were sectioned 1 hour later. Serial horizontal sections under the nipple and throughout the breast were taken to demonstrate the ductal profiles and the colored dyes they contained.

**Approach 5: Calculations of the Number and Location of Ducts and Nipple Ductal Orifices Based on the Retrospective Analysis of the Archival Ductograms of Sartorius**

In an attempt to determine the number and pattern of the ductal systems within the breast, we then analyzed 1312 archival ductograms. These ductograms had been obtained by the late Otto Sartorius, a breast surgeon in Santa Barbara, CA, in the 1960s–1970s. These ductograms represented 656 distinct ducts (2 views of each duct) in 470 women. The ductograms had been classified under Dr. Sartorius' tutelage into 10 categories based on observation. We elected to reexamine the ductograms using a different method to confirm Sartorius' classifications.

The approximate location of the center of the duct on the chest wall and the volume of the duct was determined for each ductogram by first measuring the areas in the region of the duct to either side in the two-dimensional photograph. A planimeter was used to outline the extent of the duct in relation to the total area and the areas on each side. The nipple was not included in the measurements. The back wall of the breast was defined as the rib cage, muscle, or edge of the ductogram. The back wall was chosen, which would yield the smallest area while still including the entire duct. For example, if the ductogram showed both the muscle and the rib cage, then the muscle would be the back wall if it enclosed the entire duct. If

the duct extended past the muscle wall, then the rib cage would be used as the back wall. When the ductogram did not include the muscle or the rib cage (i.e., it did not include the whole breast on the film), the total breast measurement was deemed inaccurate. In our analysis, we assumed that the breast was a hemisphere, that the cross sections were semicircles, and that the ductal shape was triangular. We divided the semicircular cross-section into three sections:  $B$  was the section of the ductogram,  $A$  was the section lateral to the ductogram, and  $C$  was the section medial to the ductogram. Using  $A$ ,  $B$ , and  $C$  from each cross-sectional view (measured quantities), we defined and calculated the following:  $x_{\text{duct}}$  = the location of the center of the duct (side-to-side view),  $y_{\text{duct}}$  = the location of the center of the duct (head-to-foot view),  $L_1$  and  $L_2$  = the lengths of the respective duct views, and  $V$  = the volume of the duct (which is a function of  $L_1$  and  $L_2$ ).

Using the measured quantities  $A$ ,  $B$ , and  $C$  from each cross-sectional view (in which the total area was normalized to a value of 1) we defined the following:  $x_{\text{duct}} = A_{\text{ML}} + 0.5B_{\text{ML}}$  was defined as the center of the duct in the craniocaudal view (measuring from side to side);  $y_{\text{duct}} = A_{\text{IS}} + 0.5B_{\text{IS}}$  was defined as the center of the duct in the mediolateral view (measuring from superior to inferior); and the relative volume of the duct was calculated to be  $B_{\text{ML}} * B_{\text{IS}}$ .

We performed a  $K$ -means cluster analysis<sup>10</sup> to determine empirically the number of ductal systems and their characteristics.  $K$ -means cluster analysis uses Euclidean distance. Initial cluster centers are chosen in a first pass of the data; then, each additional iteration group observation is based on the nearest Euclidean distance to the mean of the cluster. Thus, cluster centers change at each pass. The process continues until cluster means do not shift more than a given cutoff value or the iteration limit is reached. We clustered (grouped) the ductograms/individual ducts based on the calculated chest-wall location of their centers ( $x_{\text{duct}}$ ,  $y_{\text{duct}}$ ). The criteria of Hartigan<sup>10</sup> of large, relative reductions in within-cluster variance were used to arrive at the number of clusters. The FASTCLUS procedure in the SAS software package (SAS Institute, Cary, NC) was used to carry out the clustering computations.

**Approach 6: Confirmation of the Value of Previous Ductal Anatomy Studies in Surgical Patients with Mammographic Findings**

This approach involved selected ductal lavage of lymphazurin-identified nipple ductal orifices with mammographic correlation in six women (seven breasts) who were scheduled for surgery. This study was conducted in Chile and also was approved by the National Cancer Institute of Chile. Women who were scheduled

to undergo surgery for mammographically detected lesions were entered in the study. After informed consent had been obtained, the breast was prepped and draped in a standard sterile fashion. The nipple was anesthetized using 0.5% lidocaine. The mammogram was studied, and the lesion was localized to a particular sector of the breast. It was predicted that the involved ductal system would connect to either the central group or the peripheral group of ductal orifices. These calculations were used to identify the likely site of the corresponding nipple duct orifice. Then, the transareolar dye injection technique (see Approach 3) was employed to identify the nipple ductal orifice. Next, the orifice was cannulated (Approach 4), and a double-lumen catheter was inserted into the duct for a distance of < 1 cm. One cubic centimeter of 0.5% lidocaine was instilled into the duct to further anesthetize it, and then 10 cc of saline were lavaged. The fluid was centrifuged and stored in Cytolyte (Cytoc, Boxborough, MA), analyzed cytologically, and correlated with the final pathology.

## RESULTS

### Approach 1: Direct Observations of the Number and Distribution of Nipple Ductal Orifices by In Vitro Serial Sectioning and Three-Dimensional Digital-Image Analysis

Analysis of 10 nipples revealed that the surface contained numerous indentations, which were the sites of keratin plugs. The vast majority of these keratin plugs (> 90%) were not associated with anything in the dermis. Slightly under 10% were associated with either sebaceous glands or sweat or apocrine ducts. In 1000 serial sections (15  $\mu$  each) from these 10 nipples, 5–9 ductal orifices/nipple could be demonstrated. In these areas, very thin ducts could be seen coursing up through the dermis and emerging at a site of a keratin plug. Anticytokeratin immunocytochemistry was very helpful in highlighting these ducts and distinguishing them from sebaceous glands. The lumens of the ducts that coursed up through the nipple were much smaller (1:10–1:100) than the size of the much larger lactiferous sinuses, which lay deep to the nipple. There were no differences between the sizes and shapes of the keratin plugs that occluded the lumens of the nipple ductal orifices and those that lay over sebaceous glands or sweat ducts or stood alone. Three-dimensional digital-image analysis of the ductal locations was consistent with a central and peripheral pattern of ducts.

### Approach 2: Direct In Vivo Observations of Ductal Orifices in Lactating Women

In the lactating women, data were collected on 424 nipples in 219 women. The mean number of nipple duct orifices was 5, with an extreme range of 1–17 orifices. The patterns were symmetrical. There were

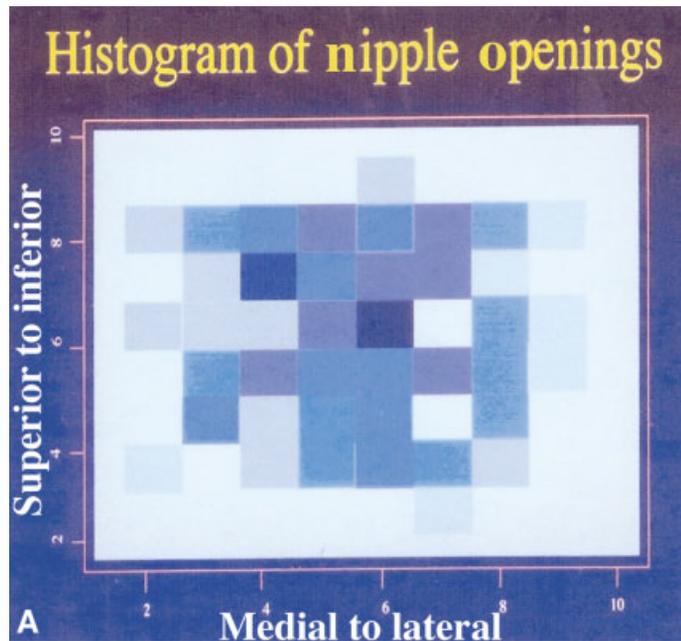
TABLE 1  
Cumulative Frequency of the Total Number of Duct Orifices in an Individual Nipple

No. of orifices	Frequency	%	Cumulative frequency	Cumulative %
1	31	7.3	31	7.3
2	27	6.4	58	13.7
3	47	11.1	105	24.8
4	60	14.2	165	38.9
5	60	14.2	225	53.1
6	50	11.8	275	64.9
7	36	8.5	311	73.3
8	37	8.7	348	82.1
9	36	8.5	384	90.6
10	16	3.8	400	94.3
11	9	2.1	409	96.5
12	7	1.7	416	98.1
13	3	0.7	419	98.8
14	2	0.5	421	99.3
15	1	0.2	422	99.5
17	2	0.5	424	100.0

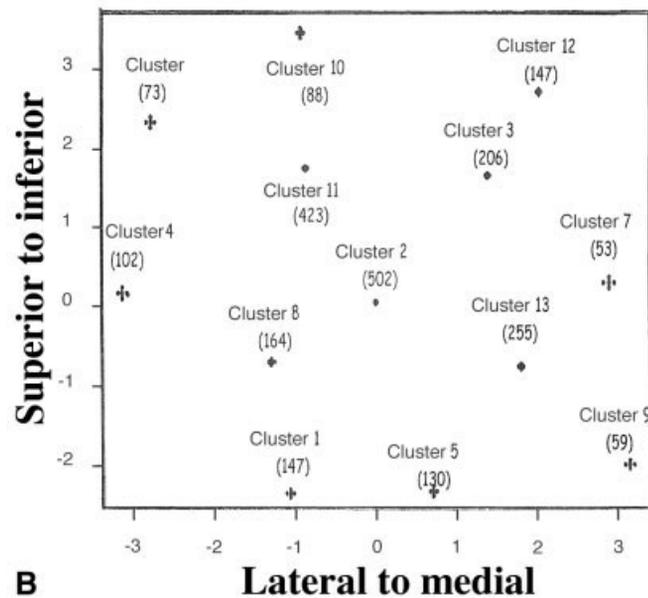
≤ 13 nipple ductal orifices in 98.8% of women (Table 1). A two-way histogram shows the relative frequency/numbers of the nipple openings by location (Fig. 1A). Darker shading indicates higher relative numbers in that region of the nipple. The most common location was in the center of the nipple. We performed a *K*-means cluster analysis<sup>10</sup> to determine empirically the number of nipple orifices and their patterns. The nipple openings grouped into 13 clusters. The mean location and standard error of the mean location for the nipple openings in each of the 13 empiric clusters are depicted in Figure 1B. Almost every woman had a duct orifice in the center of her nipple (Cluster 2) and a second duct orifice in the central area but oriented to the upper-outer quadrant (Cluster 11). The overall pattern could be described as variable, but the most consistent finding was a group of *central* orifices: one central orifice with the others just above or below the central fold. In addition, there are more peripheral openings, with upper lateral, upper medial, and lower lateral the most common. This pattern was consistent with the tear shape of the breast.

### Approach 3: Direct Observations of the Number and Distribution of Nipple Ductal Orifices by In Vitro and In Vivo Transareolar Dye Injection

In all 13 of the mastectomy specimens, the transareolar dye injection technique was effective at labeling some nipple ductal orifices. By random diffusion, the dye entered the lactiferous sinuses and wicked up through capillary action, causing dark-blue spots to appear on the nipple surface over time and marking



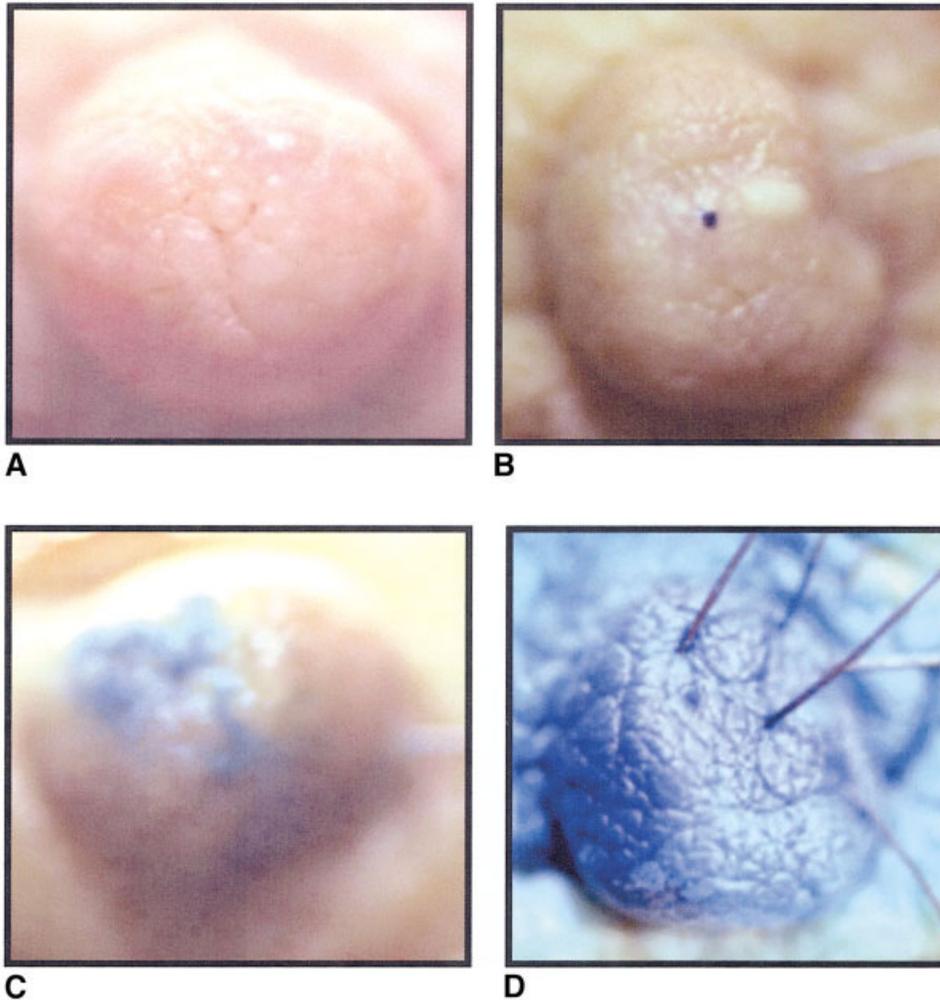
### Cluster analysis of nipple openings



**FIGURE 1.** (A) Two-way histogram shows the relative frequencies/numbers of nipple openings by location. Darker shading indicates higher relative numbers in the region of the nipple. The most common location was in the center of the nipple, and the axilla is at the left upper corner of the histogram. (B) Illustration of a cluster analysis shows the mean location and standard error of the mean location for 13 empiric clusters of milk duct orifices. The  $y$  axis represents the inferior-to-superior dimension, and the  $x$  axis represents the lateral-to-medial dimension. The numeric values shown on the axis are those used in the grid on which the orifices were mapped.

the spot of the nipple ductal orifices. The uninjected nipple was the natural-appearing nipple with a corrugated surface in which one could sometimes make out the ductal orifices (Fig. 2A). However, with this transareolar dye injection approach, the first of the first 2 ductal orifices could be identified within 10–20 seconds (Fig. 2B). The second orifice usually was identified 1–2 minutes later. These usually were the central ducts. Later (within 2–3 minutes), 3–7 additional ductal orifices appeared (Fig. 2C). These tended to be the more peripheral ducts. Eventually, the entire nipple

turned blue, but the location of all of the ductal orifices still could be discerned (Fig. 2D). In addition to coloring the ductal orifices, the injection of dye caused the nipple to be turgid and revealed orifices that previously had been hidden in folds of the nipple. There was a learning curve to this technique. Ductal orifices were identified using this technique in the first eight breasts, but not all could be cannulated and confirmed. Therefore, we could not be certain they were not sebaceous glands or sweat ducts. However, in the final five breasts, all of the ductal orifices could be



**FIGURE 2.** (A) The normal nipple exhibits a corrugated surface with numerous dimples, and the ductal orifices are not readily visible. (B) A transareolarly injected dye initially wicks up into a central duct, and (C) additional and more peripheral ducts are subsequently identified. (D) Using this technique, multiple nipple ductal orifices can be identified and cannulated with guide wires.

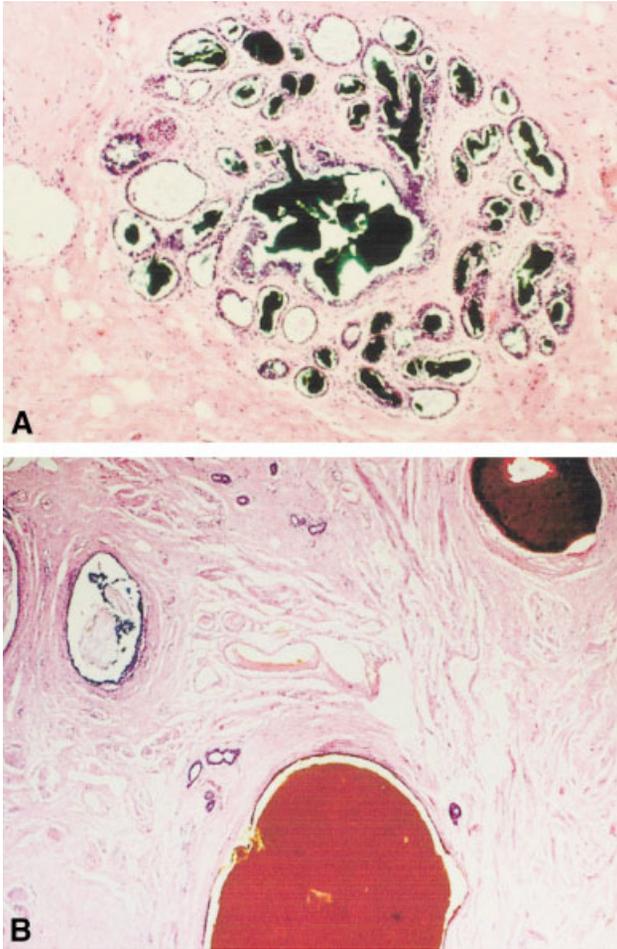
**TABLE 2**  
**Duct Identification in Postmastectomy Breasts Using the Transareolar Blue-Dye Technique**

Breast no.	No. of ducts identified	No. of ducts cannulated	No. of ducts confirmed histologically
1	5	0	0
2	6	0	0
3	3	2	2
4	2	0	0
5	3	2	2
6	3	0	0
7	4	3	3
8	5	1	1
9	7	7	7
10	6	6	6
11	6	6	6
12	8	8	8
13	7	7	7

identified, cannulated, and confirmed histologically (see Approach 4) (Table 2). The transareolar dye injection approach indicated that there were between six and eight ductal orifices/nipple. In the single patient who was studied with this approach, eight nipple duct orifices were identified without difficulty.

**Approach 4: Direct Observations of the Number, Distribution, and Physical Properties of the Underlying Ductal Systems of the Breast by In Vitro Ductal Orifice Cannulation, Dye Instillation, and Histologic Sectioning**

This study was carried out in 13 detached breasts, as described above. After initial difficulty in cannulating the ducts (Breasts 1–8), we were able to cannulate all of the ducts (Breasts 9–13) and to demonstrate histologically successful instillation of the dye, which then reached the recesses of the duct lobular units (Fig. 3A). Instillation of different dyes into separate nipple duct orifices revealed different color profiles in different



**FIGURE 3.** (A) Instillation of water-insoluble dyes in cannulated nipple ductal orifices fills one entire ductal lobular unit, a finding that provides evidence that this approach can access the deep recesses of the breast. (B) Instillation of differently colored dyes in different orifices is depicted. Although different ductal systems can appear in the same quadrant juxtaposed against one another, there is no admixing of dyes and thus no interductal communication.

ductal systems, which often coexisted in the same breast quadrant (Fig. 3B). In no case of these multiple-colored instillations was there admixing of colors, suggesting that, even when different ductal systems are in close juxtaposition, there are no communications or anastomoses among them. These different ductal systems may be misinterpreted easily as the same ductal system with routine hematoxylin and eosin staining.

#### **Approach 5: Calculations of the Number and Location of Ducts and Nipple Ductal Orifices Based on the Retrospective Analysis of the Archival Ductograms of Sartorius**

For those ductograms with the age of the patient known ( $n = 400$ ; age range, 14–88 years; median age,

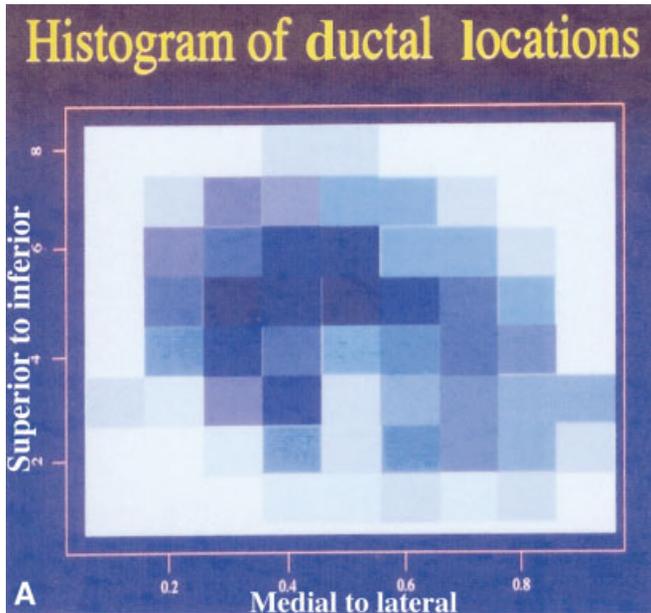
40 years), we analyzed the relation between age and  $x_{\text{duct}}$ ,  $y_{\text{duct}}$ , and  $V$ . Significant ( $P < 0.01$ ) negative correlations between age and  $y_{\text{duct}}$  and between age and  $V$  and a significant positive ( $P < 0.05$ ) correlation between age and  $x_{\text{duct}}$  were found. The significant negative correlation between age and  $V$  persisted when controlling for the effects of  $x_{\text{duct}}$  and  $y_{\text{duct}}$  on  $V$  through a multiple linear regression analysis. Similar correlations were found when we repeated the analysis choosing a single ductogram per woman ( $n = 286$  women with age known) to avoid the technical problem of within-subject correlation. The results of the distribution of the ducts are depicted in a two-dimensional histogram (Fig. 4A). This histogram shows the relative frequency/concentration of the ducts by ductal location ( $x_{\text{duct}}$ ,  $y_{\text{duct}}$ ). Darker shading indicates higher relative numbers of ducts projected in that region of the chest wall.

The cluster analysis of the ductograms was not tight. Using all 656 ductograms, there was no convergence of the clustering algorithm into  $< 20$  clusters. Using 1 ductogram per woman (selected at random), there were 11 clusters of the 474 ductograms. The mean location and its standard error of each of these clusters is depicted in Figure 4B. The general pattern of ducts in our analysis of the Sartorius ductograms indicated a central group of ducts directed toward the chest wall from the nipple and a more peripheral group of ducts extending in a more radial fashion. Other interesting findings include the fact that the ductal systems become narrower and longer with age. This undoubtedly is the result of ptosis, which increases with age and is reflected on the mammograms.

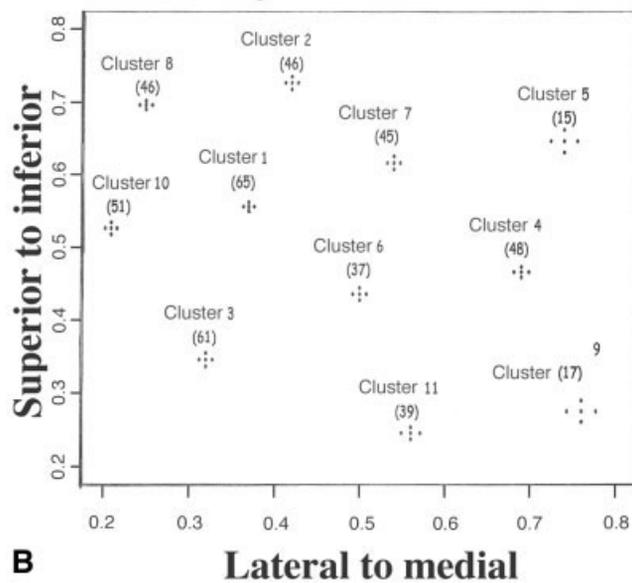
The results of our analysis of the Sartorius ductograms match our findings in Approaches 1–4. Although these approaches used different cohorts of women, and although they were not an exact match, they did corroborate certain findings. There are two groups of ducts—a central collection of ducts and ductal orifices and a more peripheral group. This overall distribution is better described as a set of concentric circles rather than as a clock face. In visualizing these ducts, it is important to remember that the breast is not two-dimensional. The ducts do not all extend in a radial fashion from the nipple; rather, some travel directly back from the nipple toward the chest wall. In addition, they vary in size.

#### **Approach 6: Confirmation of the Value of Previous Ductal Anatomy Studies in Surgical Patients with Mammographic Findings**

Our final approach was to confirm the clinical utility of this analysis. Six women were studied with no untoward events. Examining the mammogram, we located the lesion first in the inner or outer group of ducts and then in



**Cluster analysis of duct locations**



**FIGURE 4.** (A) Two-way histogram shows relative frequencies/numbers of ducts by location of the ductal center. Darker shading indicates higher relative numbers of ducts. The axilla is in the left upper corner of the histogram. (B) Illustration of a cluster analysis shows the mean location and standard error of the mean location for each of 11 empiric clusters resulting from an analysis of 1 ductogram per woman (selected at random;  $n = 474$ ). The y axis represents the inferior-to-superior dimension, and the x axis represents the medial-to-lateral dimension (with the axilla located to the left). The numeric values shown on the axis are those used in the grid on which the ductograms were mapped.

the correct quadrant. This technique allowed us to catheterize seven breasts. Of the 7 ducts that were lavaged, 4 ducts (57%) had confirmation by ductogram and mammography that the correct duct had been cannulated. In one duct, there was no dye in the breast, but some had spilled onto the patient. In another duct, the wrong duct was cannulated, and a second attempt was made without success. In the final duct, the lesion could be visualized only on ultrasound. Cytology of the lavages correlated completely with the final pathology, with three findings of benign ductal cells and one finding of malignant cells. It should be noted that three of the four successful cannulations were performed in the last two

women analyzed, hinting at the existence of a learning curve for this procedure.

**DISCUSSION**

Our findings with our multiple approaches all indicate that > 90% of nipples contain 5–9 ductal orifices distributed in 2 groups: central and peripheral. The central ducts do not extend in a radial fashion from the nipple but, rather, travel back from the nipple toward the chest wall, whereas the peripheral ducts drape over the central ducts in a radial fashion. Separate ductal systems may lie in the same quadrant in close juxtaposition to each other, but they do not connect or anastomose.

Our anatomic findings challenge conventional dogma. Most textbooks of anatomy state that there are between 15 and 20 ducts and imply that there are 15–20 nipple ductal orifices. Because of our studies, we believe that many of these orifices do not represent true ductal orifices. In 1845, Cooper<sup>5</sup> injected wax into the milk ducts in an attempt to outline their anatomy. He described being able to inject, at most, only 12 lactiferous ducts and, more commonly, 7–10 ducts. He noted, however, that there also were *tubercles* that were separate from the ducts. This finding of two different types of nipple orifices, true ductal orifices and sebaceous gland orifices, was substantiated further and expanded by Sartorius and Smith, who described their experience in > 1000 ductograms.<sup>6</sup> Those authors described two types of openings: those of true mammary ducts and what they termed secretory gland openings. They concluded that there are 5–7 true mammary duct orifices and that the other openings were sebaceous glands. These *sebaceous* glands vary in length from 1 cm to 4 cm, they have no branches, and they do not connect to the ducts. Although it is not known whether these *nonductal tubular* structures represent sebaceous glands, Cooper's wax model matched the ductograms of Sartorius and Smith almost exactly. More recently, and in a smaller series, Dietz et al.<sup>11</sup> agreed with Sartorius and Smith's observation of 5–9 ducts, stating that they were able to cannulate 2–5 ducts per specimen. Those authors went on to describe a finding that was consistent with that described by Cooper and by Sartorius and Smith.<sup>5,6</sup> Finally, Going performed an extensive 3-dimensional reconstruction of a nipple and found 27 structures with ductal appearance; however, ~0.8–1.0 mm from the nipple, most of them disappeared into a skin appendage, leaving only 7 to continue to surface through the nipple as milk duct orifices.<sup>12</sup>

Thus, one explanation for the discrepancy between 5–9 ducts and 15–20 ducts well may be the sebaceous glands/tubercles/tubes that mimic the appearance of ducts behind the nipple but do not contribute to the ductal lobular infrastructure of the breast. An additional possibility is that some ducts bifurcate shortly after emerging from the nipple. Teboul and Halliwell<sup>7</sup> described their findings in a series of > 6000 breasts that were studied through ultrasound and ductoscopy. They also described 5–8 *milk pores* but suggested that some ducts join together behind the areola to form common collectors. In our observations of nipple ductal orifices in lactating women, we also found an average of 5–9 openings in the nipple. This was confirmed further in the postmastectomy specimens, in which all of the nipple ductal orifices were cannulated and in which it was demon-

strated that the underlying ducts contained dye, thus confirming the findings of Cooper, Sartorius and Smith, and Teboul and Halliwell.<sup>5–7</sup> It is noteworthy that in all of the studies that accessed the nipple duct orifices externally, there was general agreement with the finding of 5–9 ductal orifices. All of the studies that used surgical specimens and identified the ducts subareolarly, however, suggested 15–20 orifices. This directional discrepancy may be due to either misidentifying the additional *sebaceous glands*/tubercles/tubes as breast ducts or incorrectly recognizing that a lobe is completely distinct, as described by Teboul and Halliwell.<sup>7</sup> This misinterpretation well may explain the discrepancy in the data, such as that of Ohtake et al.,<sup>8</sup> who used nipple-sparing quadrantectomies to generate elegant three-dimensional computer reconstructions of the mammary duct-lobular systems. Those authors found 16 mammary ductal systems in 1 breast and 4 with intraductal anastomoses. It is possible, however, that the ducts with anastomoses, in fact, were part of the same ductal system uniting with each other with one of Teboul and Halliwell's *common collectors* behind the nipple. Moffat and Going<sup>9</sup> used submacroscopic autopsy sections from a girl age 19 years. Those authors were able to trace 10 ducts completely that they believed represented one-half of the ductal systems present. Because they did not start at the nipple orifices, they also may have been observing the 15–20 hollow ducts identified by Teboul and Halliwell<sup>7</sup> or the sebaceous glands described by Sartorius and Smith<sup>6</sup> rather than distinct and independent ductal systems.

Another point of controversy alluded to briefly in the study by Ohtake et al.<sup>8</sup> is whether the ductal systems really anastomose with each other. Cooper<sup>5</sup> stated definitively that they did not. It is noteworthy that, in the current study, which was conducted 158 years later, we used a combination of colored dyes to support the same hypothesis. Cooper, however, did describe an intertwining of the ductal systems like the roots of a tree. It is this intertwining that makes it impossible to differentiate different ductal systems on routine histopathology and that also may account for the confusion about the number of ductal systems. In our early ductoscopy studies<sup>2</sup> and in our current work, we noted that adjacent ductal profiles often belonged to different ductal systems. Moffat and Going,<sup>9</sup> in their computer model of the ductal *catchment areas*, were able to show the overlapping of ductal systems elegantly. The assumption that adjacent ductal profiles are part of the same ductal system may be the source of erroneous conclusions about multicentricity in precancerous disease processes, such as DCIS, and in the adequacy of surgical resection margins. We found no

evidence of ductal anastomoses in our studies. If anastomoses do occur, then they probably are rare.

In most of the previous studies, the ductal systems have been described as radial. This may well result from the fact that most of the examinations have been done on breasts that have been removed and processed histopathologically and, thus, in two dimensions. Cooper<sup>5</sup> reported that some ducts radiate from the nipple, whereas others pass directly backward to the posterior surface of the gland. Teboul and Halliwell<sup>7</sup> reported a radial distribution from their ultrasound studies. Their technique involved identifying a duct near the nipple and following it with real-time ultrasound. This would necessitate compressing the breast toward one side or another, which would not be conducive to placing each ductal system in three-dimensional space. Sartorius and Smith<sup>6</sup> developed a classification system for the distribution of the ducts and analyzed all of the Sartorius ductograms according to this scheme (unpublished data). They described one duct that was centrally located and projected directly back to the chest wall. They also described central-lateral and central-medial ductal systems that projected back on either side of the central duct. In addition, there were central-upper and central-lower systems. It is not clear from their studies whether they believed that there were some ductal systems or lobes (as they called them) that projected radially. Suffice it to say, there are at least some indications from Cooper<sup>5</sup> and possibly from Sartorius and Smith<sup>6</sup> that support our conclusions regarding our independent analysis of the Sartorius ductograms.

We realize that the results of our retrospective analysis of the Sartorius ductograms have to be interpreted carefully, because there were many inherent biases. First, there was no attempt by Sartorius to catheterize a particular duct but, rather, only the duct that was most apparent. The resulting ductograms would be expected to be biased toward the ductal orifices that were more obvious or that produced nipple aspirate fluid. It is possible, then, that some of the ductal systems would not be represented in his series at all. Second, there was no standardization of mammographic positioning. This meant that a ductal system that appeared to be lateral in one woman actually may have been central in another. Taking all of this into account, we still believed that a detailed, retrospective, anatomic analysis of this series of ductograms could give us a general idea of the pattern and number of ductal systems. Using this retrospective analysis, even with its limitations, we found support for our conclusions based on our other approaches (Approaches 1–4) that the ductal systems and ductal orifices are either central or peripheral. The central ducts project

toward the chest, wall and the peripheral ducts drape around them.

Why is knowledge of ductal anatomy important? The nipple ductal orifice is the entry to the ductal lobular unit of the breast. With growing acknowledgment that breast carcinoma occurs in 1 ductal system,<sup>13</sup> we need to consider the breast as a collection of ductal systems. Preliminary studies indicate that hormone levels differ in different ductal systems.<sup>14</sup> Is it possible, then, that each ductal system represents a different microenvironment? Ductoscopy, lavage, cytology, and pathology correlation studies currently are evolving to address this question.<sup>15</sup>

Meanwhile, what we have described has many immediate, practical, clinical applications. The most obvious is in the planning of surgery. In our Chilean study, we were able to use a mammogram to predict which nipple ductal orifice communicated with the lesion, to identify the orifice with transareolar dye, and to confirm the connection with a ductogram. Thus, this technique could be used to map the ductal system preoperatively with select ductograms through the relevant nipple orifices and allow a more precise surgical procedure. Because DCIS and invasive carcinoma seem to emanate from a single duct, even in women with a genetic predisposition (i.e., *BRCA1*, *BRCA2*) to the disease, that specific duct may express a surrogate marker before there are any histologic abnormalities. Identification of a surrogate marker either in ductal fluid or in cells obtained by ductal lavage will identify the high-risk duct rather than just the high-risk patient. Accessing the right duct and the right orifice would be critical in obtaining this information. If a ductal system at risk could be identified, then specific ductal chemopreventive studies could be initiated. Directed ductoscopy, ductal lavage, and future intraductal therapy however, are all predicated on the knowledge of the correct anatomy of the ductal systems.

## REFERENCES

1. Love SM, Barsky SH. Breast-duct endoscopy to study stages of cancerous breast disease. *Lancet*. 1996;348:997–999.
2. Makita M, Sakamoto G, Akiyama F, et al. Duct endoscopy and endoscopic biopsy in the evaluation of nipple discharge. *Breast Cancer Res Treat*. 1991;18:179–188.
3. Dooley WC, Ljung BM, Veronesi U, et al. Ductal lavage for the detection of cellular atypia in women at high risk for breast cancer. *J Natl Cancer Inst*. 2001;93:1624–1632.
4. Wellings SR. A hypothesis of the origin of human breast cancer from the terminal ductal lobular unit. *Pathol Res Pract*. 1980;166:515–535.
5. Cooper A. The anatomy and diseases of the breast. Philadelphia: Lea and Blanchard, 1845.
6. Sartorius OW, Smith HS. Contrast ductography for the recognition and localization of benign and malignant breast lesions: an improved technique. In: Logan W, editor. Breast carcinoma. New York: John Wiley & Sons, 1977:281–300.

7. Teboul M, Halliwell M. Atlas of ultrasound and ductal echography of the breast. Oxford: Blackwell Science, 1995.
8. Ohtake T, Kimijima I, Fukushima T, et al. Computer assisted complete three-dimensional reconstruction of the mammary ductal/lobular systems. Implications of ductal anastomoses for breast conserving surgery. *Cancer*. 2001;91:2263–2272.
9. Moffat DF, Going JJ. Three dimensional anatomy of complete duct systems in human breast: pathological and developmental implications. *J Clin Pathol*. 1996;49:48–52.
10. Hartigan JA. Clustering algorithms. New York: John Wiley & Sons, 1975.
11. Dietz JR, Kim JA, Malycky JL, et al. Feasibility and technical considerations of mammary ductoscopy in human mastectomy specimens. *Breast J*. 2000;3:161–165.
12. Going JJ, Moffat DF. Escaping from Flatland: clinical and biological aspects of human mammary duct anatomy in three dimensions. *J Pathol*. 2004;203:538–544.
13. Holland R, Veling SH, Mravunac M, Hendricks JH. Histologic multifocality of Tis, T1-2 breast carcinomas: implications for clinical trials of breast-conserving surgery. *Cancer*. 1985;56:979–990.
14. Elia M, Handpour S, Terranova P, Anderson J, Klemp JR, Fabian C. Marked variation in nipple aspirate fluid (NAF) estrogen concentration and NAF/serum ratios between ducts in high risk women [abstract]. Presented at the 93rd annual meeting of the American Association for Cancer Research, San Francisco, CA, April 6–10, 2002.
15. King BL, Tsai SC, Gryga ME, et al. Detection of chromosomal instability in paired breast surgery and ductal lavage specimens by interphase fluorescence in situ hybridization. *Clin Cancer Res*. 2003;9:1509–1516.