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PART II

CHAPTER XII

A RESEARCH APPROACH TO VISUALIZATION OF BREAST TUMORS BY ULTRASOUND METHODS

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I. INTRODUCTION

The number of American women whose lives are jeopardized by breast cancer is large. Cancer of the breast is the main cause of all deaths among U.S. women in the age range of 40-44.[1] It can be anticipated that in 1977 there will be approximately 90,000 new cases and of the order of 35,000 deaths from this disease.[2] Further, the prognosis for normal life span after initial treatment (usually surgery) for breast cancer patients is poor.[3-9] For example, the 1972 U. S. Department of Health, Education and Welfare report on survival of approximately 25,000 patients diagnosed during the period 1955-1964 indicated a relative survival rate of 62% for the 5-year period, and 50% for the 10 year period.[10] Since survival statistics for breast cancer subjects are dependent on a complex interplay of biological and medical factors, many of which are incompletely known, caution must be exercised in interpreting such data. Nonetheless, the statistics do reveal a relationship, irrespective of the biological-medical basis of this relationship, between early detection of breast cancer and survival time.

Clinical statistical data indicate that the most important factor in regard to long-term survival of the breast patient is the extent of the disease at the time of surgery, i.e., whether the cancer has metastasized to regions beyond the breast.[3-6,11] Since the size of the tumor is related to the occurrence of metastases, it can be postulated that long-term survival of breast cancer patients would be improved if breast tumors were detected while they are still of small dimension. The data of a number of investigators confirms this assumption.[5,6,12]

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It is not being suggested here that detection and subsequent removal of either the mass or the whole breast will insure a completely normal life span for the patient. The studies of both earlier and more recent investigators reveal that breast cancer has a continuing deleterious effect, over the lifetime of the patient, insofar as life span is concerned, despite total excision of the breast.[5,7-9] Nonetheless, this does not obviate the value of considerably increasing the life span of that large population of women who presently harbor small malignant masses within their breasts by detecting these masses prior to spread of the malignant cells. This is particularly true in view of the present poor success in detecting breast tumors while they are still of small dimension. For example, in the United States, in approximately 95% of confirmed cases of breast cancer, the patient originally detects the breast mass and in approximately 60% of these cases the malignancy has already spread to the axillary lymph nodes, a stage where 5 year survival is of the order of 40-45%.[2,4,8] Obviously, the tumors are, on the average, of large dimensions by the time the patient feels the mass. In fact, in a 1969 publication, Ross[13] indicated that breast tumors are of the order of 5 cm in average diameter when first discovered by the patient.

As indicated by the above brief discussion, there is clearly a serious need for additional breast cancer detection techniques. Such new methods must be non-injurious to the patient, with no cumulative effects from repeated use. Most important, the technique should be capable of differentiating, without ambiguity, between benign and malignant tumors and, in addition, should be capable of detecting such tumors in early stages of development. Ultrasound visualization methods can fulfill the above requirements if sufficient research is carried out to provide certain information, which is not now known, regarding the interaction of ultrasound and breast tissue structures.

The potential of ultrasound visualization techniques for general clinical diagnosis was initially demonstrated by use of this method for breast tumor detection. During the 1950's, two separate groups, Wild and associates and Howry and his co-investigators,[14-18] carried out the first studies on the use of ultrasound visualization for the study of the breast. Wild and Reid, using 15 MHz frequency sound, obtained two-dimensional echograms of in vivo breasts which clearly showed such phenomena as echo-free, liquid filled cysts and dense echo producing malignant tumors. These early investigators indicated the necessity for solving many instrumentation problems before the ultrasonic technique could be realistically used as a clinical tool, especially in regard to the clear differentiation on the echograms of the differences between benign and malignant tumor patterns. Howry, in particular, pointed out specific problems associated with true identification by ultrasound techniques, of anatomical structures with complex geometry or steep angular borders and the type of identification errors resulting from poor axial or azimuthal resolving power of the system. Although individual investigators in various parts of the world carried out subsequent investigations which expanded knowledge regarding the potential of ultrasound for diagnosis of breast disease well beyond that of the initial work of the 1950's, ultrasound was not routinely clinically applied for detection of breast cancer, with the exception of the clinical investigations in Japan.[19,20]

In the United States, at the present time, while ultrasound visualization techniques are providing significant medical diagnostic data for a variety of body structures (especially abdominal organs and the heart) and, further, while commercial ultrasound instrumentation is readily available for such purposes, there is no such comparable situation in regard to breast examination. In this past decade of proliferation of clinical ultrasound instrumentation, despite the high incidence of breast cancer in the United States and the need for additional detection methods, commercial ultrasound instruments in the United States were not specifically designed for breast scanning.

There are many reasons for this lack of progress in regard to the ultrasound visualization of breast tissue, including the fact that the breast, in comparison to other body structures, represents a complex path or medium for the high frequency sound wave traversing it. Further, this "medium" exhibits great variability from subject to subject within the same age range, as well as variability with the aging process itself. There were other, rather practical bio-engineering problems which accounted for some of the lack of progress in this area of clinical ultrasound but these will not be discussed here.

Most important to this overall problem is that breast carcinomas have, macroscopically and microscopically, a large spectrum of variability. A critical question, therefore, that faced all individuals contemplating the use of the ultrasound techniques for breast examination was whether the different types of malignant breast tumors had characteristics, in relation to a high frequency sound beam, which would allow them to be differentiated from the many types of solid benign tumors. It is of interest in that regard to consider the work of various investigators whose studies have some relation to this basic question.

Fukuda and Wagai,[21] in the 1964 Annual Report of Juntendo University School of Medicine, reported results obtained by examination of 170 breast patients with B-scope instrumentation using sector scanning and a 5 MHz unfocused transducer. A most important aspect of the investigations included in this report were the attempts to define the characteristics of the echogram pattern of malignant tumors. To that point, the general observation was made that when localized, irregular, intense reflections are detected within the breast, these reflections are an indication of the presence of a carcinoma. It was further observed, however, that if a cancer is relatively large (not defined), the interior of the tumor, rather than being highly reflecting, is echo-free with essentially no reflections received from within or from the distal border of the tumor. As the authors point out in this report, attenuation of the ultrasound must take place in the malignant tumor to an extent that insufficient sound penetrates the tumor to allow reflections from the distal border.

It is significant that Wagai and associates, at this stage of their studies, found two opposite results for malignant breast tumors. In some there was an intense, localized reflection from within the tumor, and in others a condition of essentially no echoes from the interior regions of the mass. Clearly, such results must be intimately associated with basic tissue structure; unfortunately, no information was provided in the publication as to the specific histological structures of the malignant tumors pictured.

A brief summary of the progress of the ultrasound breast studies appearing in the 1970 Annual Report of Juntendo University School of Medicine includes three categories of echogram patterns for malignant breast tumors. In addition to the two described in the 1964 report, a third type was reported that resembled the pattern of benign tumors to the extent that the inferior border of the tumor could be defined and the interior of the tumor was not highly reflecting.[22] The differentiating characteristic of this type of pattern was its irregular border in contrast to the smooth boundaries of benign tumors. This third classification of echogram pattern for breast malignant tumors was apparently first discovered by a group of investigators at Gunma University School of Medicine in Maebashi, Japan.[23] Studies were carried out by these authors on the relation of the three echogram patterns to the macroscopic and histologic characteristics of the tumors. No relationship was found between diameter of the tumor and ultrasound pattern but the preliminary findings did indicate a possible relation to histologic classification.

A publication by Japanese investigators from Osaka City University Medical School also indicates the possibility of relating echogram pattern to histological classification.[24] In a recent review of the ultrasound clinical studies carried out at the Medical Ultrasonics Research Center of Juntendo University, Dr. Wagai[25] places primary emphasis, insofar as the breast studies are concerned, on the finding that benign tumors show less attenuation than surrounding normal tissue while malignant tumors demonstrate greater attenuation, particularly at the higher frequencies.

The investigations of E. Kelly Fry and associates[26-38] showed the advantages of a research approach to breast tissue visualization, including the use of a computer assisted visualization system in combination with wide aperture, focused transducers and sensitive amplifier systems. Included in the results were the recognition of benign tumors, such as fibroadenomas, by the fine echo pattern from within the tumor; the attenuation characteristics of some malignant tumors; and the detection of certain disseminated conditions, such as fibrosing adenosis by a quantitative approach which compared the overall echo patterns of such conditions with those of normal subjects of the same general age range.

The breast studies of DeLand[39] are of interest from the viewpoint that he found that malignant tumors classified as scirrhous adenocarcinomas gave echogram patterns showing irregular borders and echoes from within the tumor, while those classed as medullary adenocarcinomas showed regular margins with no echoes from within the tumor. Some scans were made on surgically removed breasts and compared with breast scans of the same patients prior to surgery.

Scientists from the Commonwealth Acoustics Laboratory in Australia have reported that (1) malignant tumors less than 2 cm in diameter have a low level of echoes from internal regions, jagged boundaries and exhibit significant attenuation of ultrasound, (2) large malignant tumors do not attenuate the sound, (3) for the single case of mucoid carcinoma studied, the internal echo pattern is similar to normal tissue and the tumor does not attenuate the sound beam.[40]

This chapter reviews an early interdisciplinary approach taken by the authors in an attempt to gain insight into this complex problem. Specifically, tumor structure as revealed by ultrasound visualization of formalin-fixed, excised breasts was correlated with tumor structure as determined by subserial whole breast sectioning and staining by histologic techniques.

2. BACKGROUND

The usual procedure in the case of a patient with a suspicious mass or other abnormality within the breast is surgical excision of the tumor followed by immediate frozen section examination. If cancer is diagnosed, mastectomy is carried out at the same surgical procedure and both gross and microscopic examination of the breast and tumor follow. The purpose of this examination, in the usual clinical setting, is to verify the diagnosis of cancer, to evaluate prognosis and to establish indications for adjuvant therapy. Conventionally, the primary mass itself and the axillary lymph nodes, which provide data on the spread of the disease, are the structures receiving most attention. The bulk of the mammary tissue is sampled sparingly, if at all. The average size of a section is on the order of 1 x 1.5 cm.

While examination by such methods suffices for clinical purposes, it is less than satisfactory as a basis for evaluation of techniques designed to image the intact breast. Not only is distortion introduced by the biopsy procedure, but the sampling error is clearly a totally uncontrolled variable. The histological technique of whole-breast sectioning and staining developed by Gallager and Martin,[41,42] and used by them in studies of information contained in mammograms of breasts with malignant tumors, obviates most of these difficulties. The procedure applied is outlined below under Methods of Procedure.

The information revealed by such whole breast histological studies is extremely valuable for interpretation of ultrasound visualization results and for providing the information necessary for accelerated advancement of the ultrasound method for the study of breast diseases. If, in fact, patients could be ultrasonically scanned prior to their mastectomies and comparisons made between the echograms and histological sections for the same cross-sections of tissue, this would be an ideal experimental plan. Discussions were held between the two authors and associates regarding the possibility of carrying out such an approach. Unfortunately, it was not feasible to bring the patients from M. D. Anderson Hospital in Houston, Texas to Interscience Research Institute in Champaign, Illinois, where the ultrasound research instrumentation was located. Further, since the instrumentation was a sophisticated, computer based system,[26] it could not be easily transported to the medical facility in Texas. Consideration was given, therefore, to the possibility of ultrasonically scanning the formalin-fixed breast specimens of the M. D. Anderson Hospital patients since these tissue samples could be transported between the two research facilities without undue difficulty.

In considering the changes produced by formalin fixation, it is of interest to note that Yukishita, et al.[43] found that the acoustic impedance of both normal and abnormal brain tissue was only slightly changed by formalin fixation. Encouraged by these results, and the then ongoing studies of one of the authors on acoustic visualization of fresh and formalin-fixed human liver, the following experiment was undertaken in the case of one patient who was scheduled for a surgical biopsy and probable mastectomy in a Champaign, Illinois hospital as a result of a diagnosis of breast cancer by clinical and mammographic examination. The purpose of the following described experiment was simply to determine whether the echogram pattern of breast when freshly excised and after formalin fixation was essentially comparable to that of the in vivo breast and whether the formalin fixation produced drastic changes in acoustic impedance as judged by pulse echo visualization techniques. Since it was known that the patient would undergo the standard procedure of removal of the primary tumor during the surgical biopsy with the result that the excised breast would not contain the previously ultrasonically detected tumor, study of the changes produced in the malignant mass by the formalin fixation process was not part of this experiment.

The subject available for this procedure was 66 years of age with very large (brassiere size 42D), fatty breasts. If a breast is of large volume, floating, and the sound beam is directed across the anterior surface, considerable depth of penetration is required for the examining sound beam. Therefore, for this subject, the lower frequency transducer (1.7 MHz) was selected. The patient was scanned, prior to surgery, by a technique of direct water coupling between transducer and skin with the breasts floating and the patient supine.[31,44]

Immediately following the mastectomy, the excised breast was placed in mammalian saline solution, transported to the ultrasound laboratory and scanned within three hours following surgery. The scanning was carried out under precisely the same instrumentation conditions used for the pre-surgery scanning, with the breast in a thin plastic bag filled with mammalian saline. A special effort was made to extrude all air from the bag. The bag with enclosed breast was immersed in a 37°C saline bath which contained the examining transducer. The excised breast sample was then scanned in the same regions and with the same general procedures as had been used for the *in vivo* scans. Following the completion of these scans, the excised breast was immersed in a large 10% formalin bath maintained at room temperature. After three days of tissue immersion, the breast sample was placed in a plastic bag in formalin solution, the air was extruded, the bag immersed in a 37°C saline bath and the same scanning procedure carried out for the freshly excised breast was applied; after 6 days of formalin fixation, the scanning procedure was again repeated. No significant changes were detected between the 3- and 6-day intervals.

As indicated, the subject of this examination was large breasted and post-menopausal. The breast tissue, therefore, was predominantly composed of fat interposed with connective tissue. These structures could be visualized at the same attenuation settings for the *in vivo*, freshly excised and formalin-fixed specimen with no visually detectable changes in echogram information. At very high attenuation settings, such as those in which only the skin line and a few highly reflecting structures were visualized, less echoes were seen *in vivo* than in the excised breast, whether in saline or formalin fixed. This phenomena, however, may have been partially due to difference in viewing angle. *In vivo*, with the breast floating, the skin surface is at a sharper angle than in the excised tissue situation. The general conclusion from these studies was that since the acoustic impedance of the fatty, post-menopausal breast was not significantly changed by formalin fixation for the time periods indicated, (as judged by visual inspection of echograms obtained at 1.7 MHz frequency) there was strong justification for attempting to scan the unique formalin-fixed breast specimens with intact malignant tumors from M. D. Anderson Hospital. It was not assumed that because the acoustic impedance was apparently not changed to any significant degree there were not significant changes in the tissue as a result of the formalin fixation.

It was also judged that if there were continuing, significant changes in acoustic impedance with long-time formalin fixation, these alterations could be recognized in studying the specimens from M. D. Anderson Hospital which would have been formalin-fixed for at least a number of weeks. Drastic differences in results from visualization of these specimens in comparison to visualization of the intact breasts of subjects in the same general age group would have been recognized, since the same instrumentation and transducers would have been applied in both cases.

The reasoning associated with the decision to attempt ultrasonic scanning of formalin-fixed tissue was not based only on the fact of the difficulty of scanning the specific patients described above. The experimental design included the premise that if formalin fixation did not seriously alter the acoustic impedance values of breast tissue, then examination of formalin-fixed tissue by visualization techniques would be a valuable research method. It would allow detailed study of selected breasts over long periods of time, a variety of types of transducer designs and instrumentation systems and, in addition, would make feasible comparative studies of the same pathological breast by several ultrasound laboratories.

3. METHODS OF PROCEDURE

The overall approach and general procedures associated with the study presented in this chapter were as follows. The patients selected were women with discrete palpable mass lesions of moderate size (1 to 3 cm) in whom x-ray mammography yielded definite diagnosis of carcinoma. The histologic diagnosis in each case was established by Trocar needle biopsy and frozen section. This procedure removes from the mass a slender cylinder of tissue 0.1 cm in diameter and 1 to 2 cm long. The mass itself, therefore, remains intact in its intramammary relationships except for the presence of some minor hemorrhage around the needle tract.

Mastectomies were performed by standard surgical techniques. The most commonly employed procedure was modified radical mastectomy, in which the breast, a sizable ellipse of overlying skin including the nipple, the axillary contents and the pectoralis minor muscle are removed in continuity, but the pectoralis major muscle is left. In the pathology laboratory, the muscle and axillary nodes were first removed and prepared for histologic examination by conventional techniques. The breast itself was divided into three parallel blocks, 6-8 cm thick by longitudinal incisions. Each block was molded in a shape conforming as closely as possible to that shown on the mammogram of the breast taken prior to surgery and finally fixed in position in 10% neutral formalin solution. These formalin-fixed blocks were then transferred from M. D. Anderson Hospital in Houston, Texas to Interscience Research Institute in Champaign, Illinois for detailed ultrasound scanning.

The tissues were maintained in 10% formalin solution at all times except during the scanning procedures when they were immersed in degassed saline. Following ultrasound visualization, as detailed below, they were returned to M. D. Anderson Hospital where they were paraffin embedded and sectioned. Sections 10-15 microns thick were taken at intervals of 1 mm, stained and microscopically studied. Details of the histological procedures are provided in Gallager and Martin's[41,42] publications. Each block of breast tissue provided a set of 5 x 7 inch slides ranging in number from 70 to 100. All of the breast tissue from skin to base of the breast were represented in these slides. Since the ultrasound visualization echogram represented a thin, approximately 1 mm, cross-sectional slice through the depth of the breast and, in that regard, is similar to a histological cross-section, direct comparison of the echogram data and the histological data could be made.

More than one type of ultrasound transducer was used to study the excised breast tissue but the primary data presented in this document was obtained with a computer-controlled, B-mode visualization system, used in conjunction with 9 cm diameter, 20.5 cm focal length, lead zirconate titanate disk transceiver with a frequency of 1.7 MHz, or a 5 cm diameter, 10 cm focal length, lead zirconate titanate transceiver with a frequency of 2.4 MHz. Included in the electronic system was a variable control unit which attenuated (prior to its input into the amplifier) the electrical signal generated by the tissue echo impinging on the transducer. This unit was, in essence, a gain control. The variable settings were presented in db units with a high db indicating increased attenuation of the signal and, therefore, low gain for the system. The attenuation factor, or db setting, is significant to the results obtained. If a low value is used, the strong signals received from highly reflecting targets are only mildly attenuated and this may prevent differentiation on the echogram of less intense reflections from adjacent structures of different acoustic impedance. If, however, the attenuation value is set too high, small amplitude reflections are completely undetected. Earlier publications discuss further specific details of the computer, acoustic and electronic aspects of the system.[26,31] Polaroid photographs of the information presented on a CRT were used to record the breast scan information.

Initially the transducer was positioned over the anterior surface of the breast at a distance which would place the focus (Z-coordinate) about midway in the tissue. The placement of this focus was varied during the procedures. The transducer motion was a sector sweep, starting with the transducer in a predetermined position in relation to the tissue (linear X-coordinate), the sweep was initiated by the transducer pivoting about its axis for an angular sweep motion of $\pm$ 15 degrees. With each sector sweep of the transducer, data for a maximum linear distance of 9.5 cm of tissue was recorded at a 1:1 magnification by Polaroid photography of the CRT. With the aid of the computer, after each single scan, the transducer was automatically moved in 2-5 mm linear steps (Y-coordinate) and another scan was recorded. This method of operation was continued until, essentially, the complete specimen of tissue was scanned. The X, Y, Z coordinates were automatically recorded on each Polaroid. For a longitudinal scan, the transducer was positioned so that it swept the tissue along the superior-inferior axis of the breast. For transverse scanning, the transducer was turned 90 degrees from the position set for longitudinal scanning, i.e., the transducer moved across the medial-lateral aspect of the breast.

In order to relate data recorded on the echograms to specific areas of the tissue, an antomical landmark on the tissue (such as the center of the nipple or the center of a prominent skin discoloration over the area of abnormality) was selected and a highly reflective and attenuating acoustic target placed on this landmark. With the focus of the transducer set on the target center, the echogram of the breast tissue showed a distinct, easily identified front surface reflection and an attenuation shadow of the target. Since the linear coordinates (X and Y) were recorded on each echogram, subsequent scans of the tissue with the target removed could be directly related to the chosen anatomical landmark.

In order to relate the information on the echogram to the gross tissue sample and subsequently to the histological cross-sections, photographs (1:1) were taken of the gross tissue samples, which included all reference features of the tissue, such as center of the nipple or distinctive skin markings. An overlay drawing was made of this photograph and used as a reference data record of the location of the transverse and longitudinal scans of the tissue (see Figures 1 and 2). Although the transverse scan data was important for detection, it was not necessary for the correlations with histology findings since the breast tissue was sectioned along the superior-inferior axis for the histological study.

The above outlined procedure is essentially a search technique for the purpose of (1) identifying on the echogram the normal components of the tissue under study, and (2) recognizing any pathological structures within the normal framework. However, in addition to this search technique, detailed

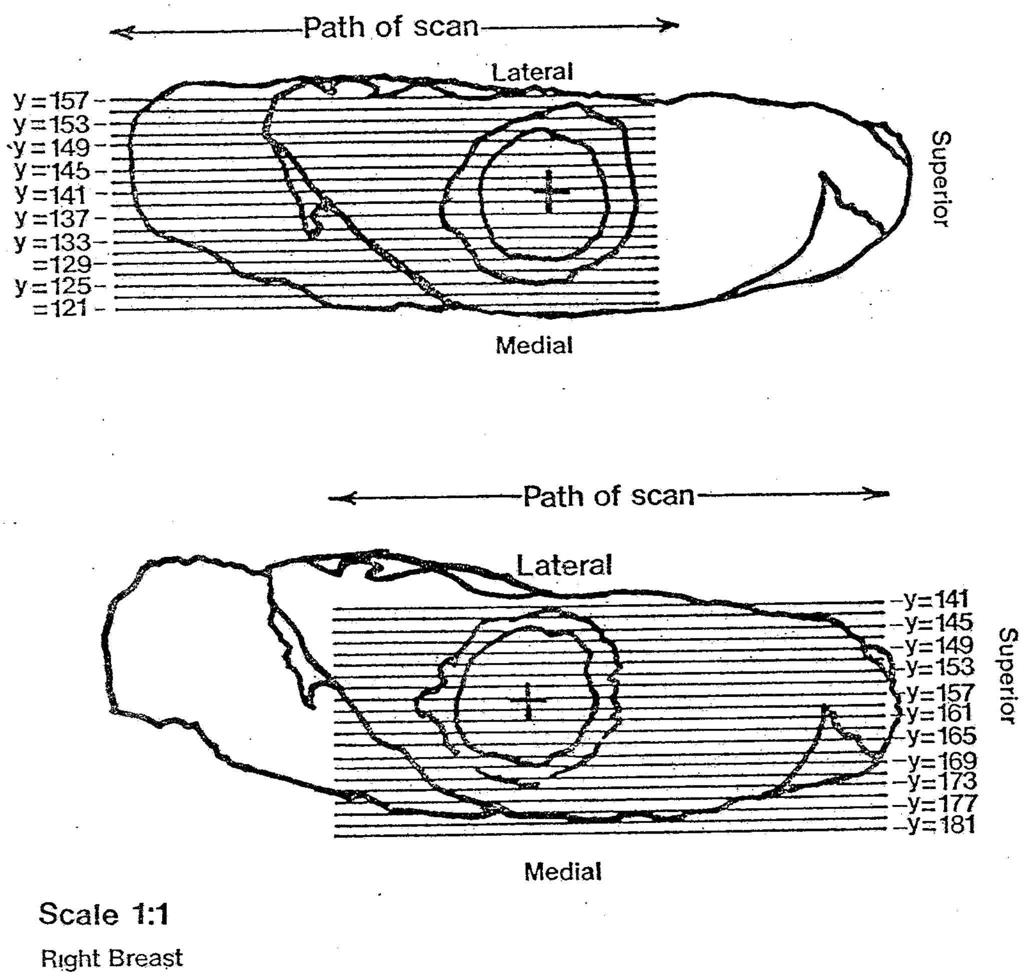
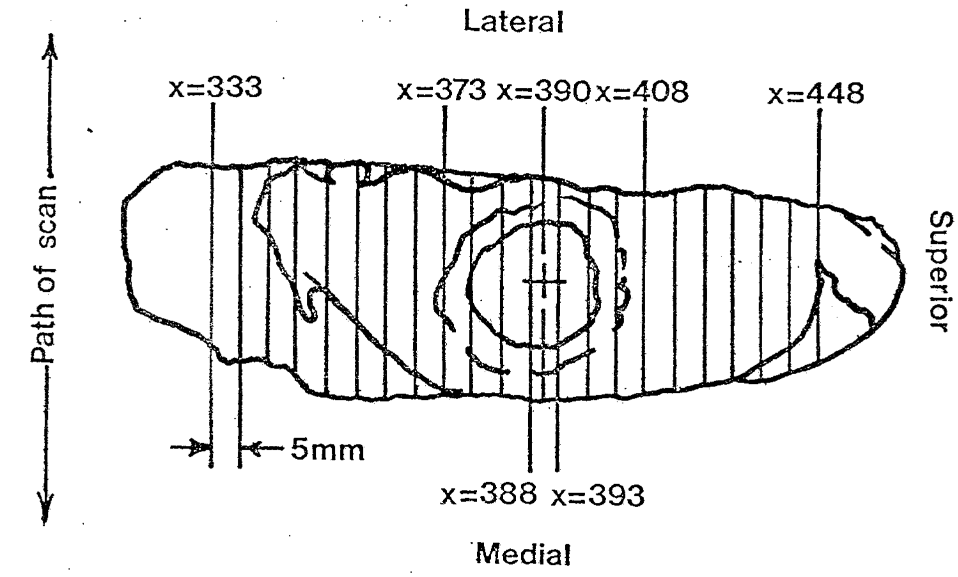


Fig. 1 Data record showing the planes of the longitudinal scans on an overlay drawing of the anterior surface of in vitro breast specimen.

scans were carried out in specific regions of the breast tissue which had been identified as malignant or suspicious by the pre- or post-operative mammogram or pre-operative clinical examination. The data obtained from the mammograms was presented in the form of a 1:1 sketch of the breast tissue specimen with regions of suspicion clearly marked. These areas were then examined in detail.

Results obtained on four breast specimens are included in this chapter to illustrate the advantages of this approach to the problem of correlating



Right Breast
Scale 1:1

Fig. 2 Data record indicating the planes of the transverse scans on an overlay drawing of the anterior surface of in vitro breast specimen.

the structure of the malignant tumor and the echogram pattern. Several hundred individual echograms were recorded for each breast specimen.

4. RESULTS

The first breast scanned in this series was the right breast of a 53-year-old Latin-American female two years past a spontaneous menopause. Clinical examination of this subject prior to surgery showed that both breasts were large, fatty, atrophic and pendulous. There was a central mass fixed to the skin of the areola of the right breast which clinically measured slightly less than 3 cm in diameter. This specimen was ultrasonically examined, using the 1.7 MHz frequency transducer according to the procedure outlined under Methods of Procedure. Figures 1 and 2 show typical data records relating the X, Y coordinates of the transducer position to the location of the scan in the tissue as shown by a 1:1 drawing of the anterior

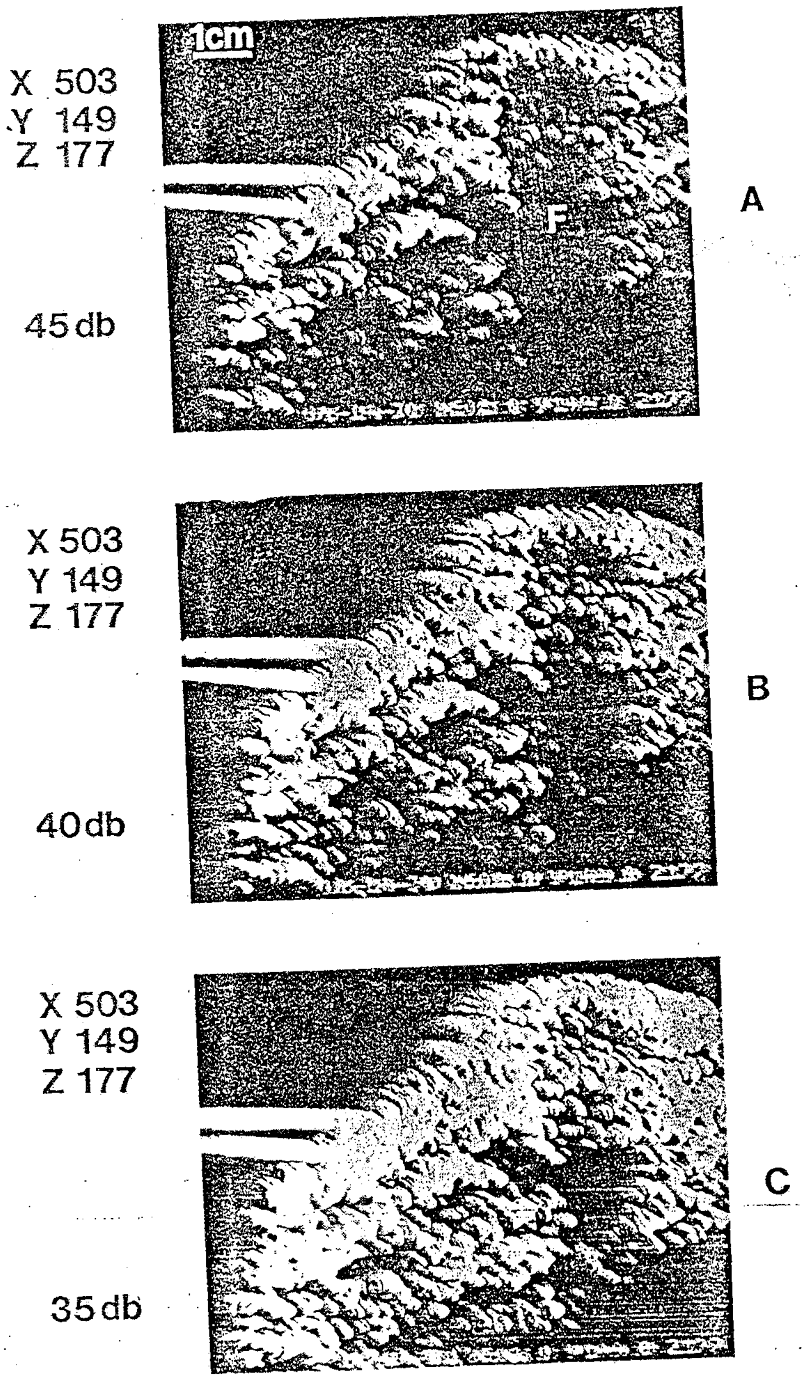


Fig. 3 Echograms obtained by longitudinal scans, at different instrument gains, of formalin-fixed in vitro breast in region of malignant tumor.

view of the breast specimen. One or more echograms were recorded for each scanning line indicated on the drawings (for different values of gain and placement of the focus). No significant results were found in any regions other than the tissues just below the nipple and areola.

Figures 3a, b and c show three echograms of longitudinal scans across the areola obtained at the instrument attenuation values shown as a db designation on each echogram. The precise location of the region of the scan for these echograms can be determined by noting the scan line labeled Y - 149 in Figure 1. As is evident from Figure 3a, attenuation of the sound in the region distal to the areola is clearly evident. However, approximately 1.5 cm below the skin, low intensity reflections are recorded from a structure about 1.5 cm in diameter. With a 5 db change in instrument attenuation, as shown in Figure 3b, reflections are recorded from the tissue beneath the areola but the attenuation phenomena is still evident by the shadow just distal to the 1.5 cm diameter structure faintly visualized in Figure 3a. A further 5 db change as shown in Figure 3c results in increased intensity of reflections in the region below the areola, but the shadow due to the attenuation of the sound is still present. The focus placement (F) in all three of the echograms shown in Figure 3 was in the region of the shadow. The focus was also placed within the mass with no essential difference in the results. The attenuation shadow was recorded for a 3 cm distance along the superior-inferior axis.

Figure 4 is a black-and-white duplication of one of the stained histological sections obtained of the region of the breast shown by the echograms of Figures 3a, b and c. In the original stained histological section, the small areas of necrosis and fibrosis within the sub-areolar tumor can be easily differentiated histologically and by their individual staining characteristics. In Figure 4 the fibrotic area is shown as a small region of lighter gray, and the necrotic tissue is indicated by the small, dark dot-like structures, the result of fragmentation in sectioning. In terms of Gallager and Martin's[41,42] classification of invasive masses based on the subgross

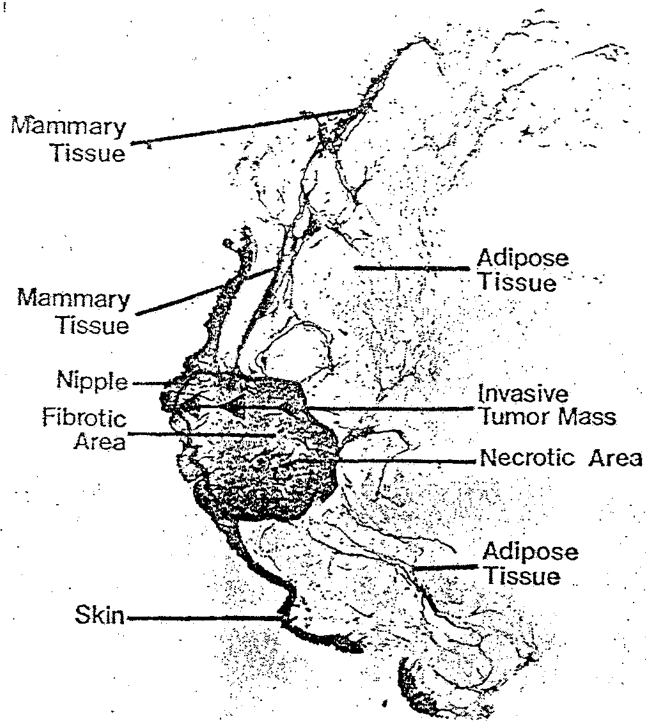


Fig. 4 Histological section of whole breast specimen with large malignant tumor. Echograms of this breast specimen shown in Fig. 3.

characteristics, the mass would be categorized as "knobby." Such tumors are spherical or ovoid with smoothly rounded protuberances on the periphery; the advancing edge of the mass is histologically sharp and is either separated from the surrounding tissue by a narrow band of collagen or is in direct contact with it. In masses of this type which are of the order of 3 cm or more in diameter, central necrosis often occurs and may include cyst formation. The centers of knobby carcinomas are usually fibrotic, while the peripheral areas are intensely cellular. The cells are usually arranged in solid masses or nests, but an occasional tumor may show glandular or ductal differentiation.

Figures 3a and b apparently indicate that an attenuation phenomena is associated with the complete malignant mass, rather than just the central fibrotic and necrotic regions. It is significant, however, that the greatest attenuation is in the fibrotic, necrotic region as shown by the echograms of Figures 3b and c. In regard to delineating the regions of attenuation, within the tumor, there is a unique factor associated with this specific tumor, described here, namely, that it is located just below the nipple-areola region. Apart from the question of the structural composition of the tissues in this region (ductal structures and glandular tissue) in comparison to other regions of the breast and the variation of this structural pattern with age, the significance of this location of the tumor is the possible attenuating characteristics of the nipple. One of the authors of this chapter (E.K.F.) found attenuation of the sound in the region of the nipple for the normal breast, as judged by ultrasound visualization. There is also some attenuation in the areolar region but this is considerably less. Similar results were found in studies of formalin-fixed breasts and it was found in these specimens that, as expected, the attenuation was frequency dependent. Consideration must be given, therefore, to the effect of this nipple-areola attenuation on the results shown in Figures 3a, b and c. The sharp demarcation of the attenuation shown in Figure 3a, the low frequency of the sound beam (1.7 MHz) and the fact that the attenuation was evident over the whole region of the areola and not confined to the more attenuating nipple region, leads to the conclusion, in this case, that the cellular mass is attenuating. The echograms obtained at greater sensitivity (3b and c) clearly demonstrate that the attenuation shown in the deeper regions of the tumor is not a function of attenuation by structures in the region of the skin line.

In view of the above findings the results obtained with one of the other breast specimens in this series, also examined with the 1.7 MHz transducer, are of special interest. It was known prior to the ultrasound scans that the specimen contained a 2.5 cm malignant tumor and the general location of the tumor was provided by the specimen radiograph. Nonetheless, despite detailed scans in both the transverse and longitudinal mode, the tumor could not be located with absolute certainty. There were two reasons for this: (1) attenuation was present but not sharply defined in the presumed tumor

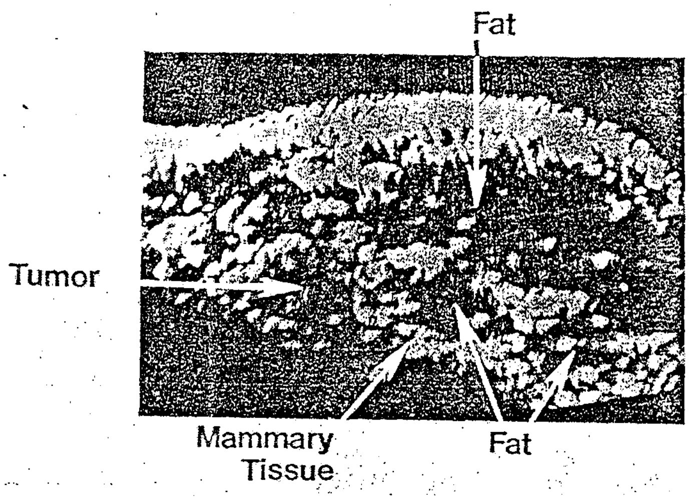


Fig. 5 Echogram obtained by longitudinal scan of formalin-fixed _in vitro_ breast in region of malignant tumor.

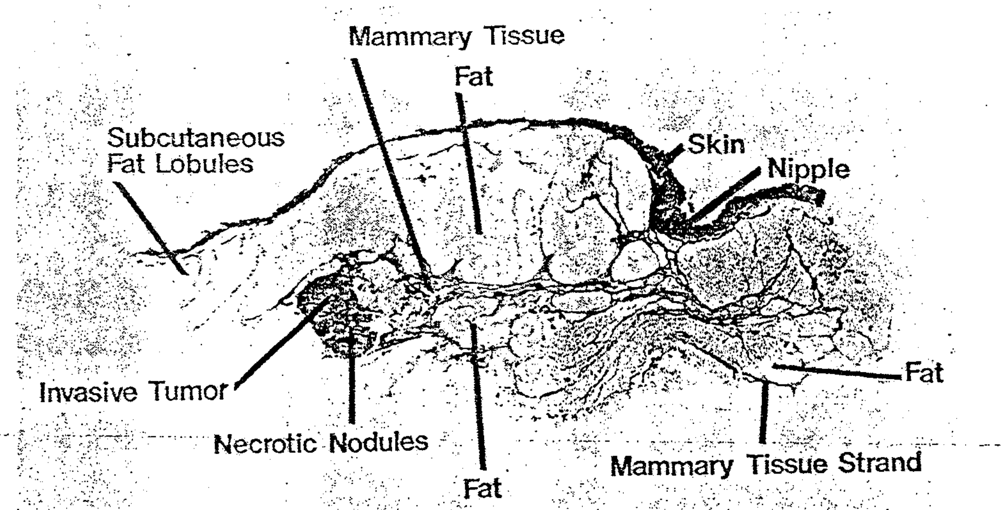


Fig. 6 Histological section of whole breast specimen, with deep invasive tumor. Echogram of this breast specimen shown in Fig. 5 above.

region, that is, some structures were visualized through the area, and (2) a non-attenuating, walled structure of approximately the same size as the tumor and located immediately adjacent to it was identified on the echogram. Figure 5 is a duplication of one of the echograms showing both of these structures. In view of the nebulous attenuation and the distinctness of the walled structure, there was serious doubt about the identification of the tumor region. In attempts to resolve this problem, some linear scans were made of this specimen, using a relatively simple laboratory scanner and the same transducer. The linear scans more clearly defined the attenuation region but the result was generally the same, that is, the presumed tumor had a low grade attenuating characteristic.

The histological findings on this specimen are significant in clarifying both the structural character of the malignant tumor and the adjacent tissue structure which gave the appearance of an encapsulated mass. The mass identified on the echograms as mildly attenuating was classified as invasive carcinoma of the knobby type, but with no solid, clearly defined necrosis and only a small (0.5 cm) central fibrotic focus. There were small, dispersed necrotic foci within the mass but these did not form a coalescent mass. The adjacent walled structure observed on the echogram was found on the histological sections to be a mass of fat, loculated by residual mammary tissue. Figure 6 is a duplication of one of the histological sections and clearly shows the tumor and the adjacent fat structures surrounded by the mammary tissue.

In considering echogram patterns of middle-aged breasts, it should be remembered that atrophy of the ducts and lobules (these are the epithelial components of the mammary tissue of the mature, young breast) accelerates after menopause and that the supporting fibrous tissue of the breast participates in this process and is gradually replaced by adipose tissue. As a result, mammary tissue in the post-menopausal breast consists of thin overlapping plates of periductal collagenous connective tissue, which contain residual epithelial structures and are separated by islands of fat of varying size and shape. There is great variability in the rate and extent of this process of atrophy and replacement depending upon, among other things, length of menstrual life, number of pregnancies and nutritional status.

Another specimen examined was from a 65-year-old subject whose preoperative mammogram revealed a 2 cm mass located deep in the lateral aspect of the left breast. For this specimen the 2.4 MHz transducer was used. There was no difficulty in identifying the tumor because it was distinctly attenuating. Figure 7 shows the result of a single scan in the region of the tumor. The histological sections for this specimen are of considerable interest because they showed that the breast malignancy in this subject consisted of a discrete knobby mass of duct cell carcinoma approximately 1.5 cm in diameter overlying an irregular, comma-shaped nodule of invasive lobular carcinoma. Figure 8 is a black and white duplication of one of the histological sections.

In view of the position of the duct cell carcinoma and the fact that it was attenuating, it is not possible, for this specimen, to define the echogram characteristics of the lobular mass. Figure 9 shows another echogram of this same specimen in which an outline of the duct cell carcinoma mass is imaged.

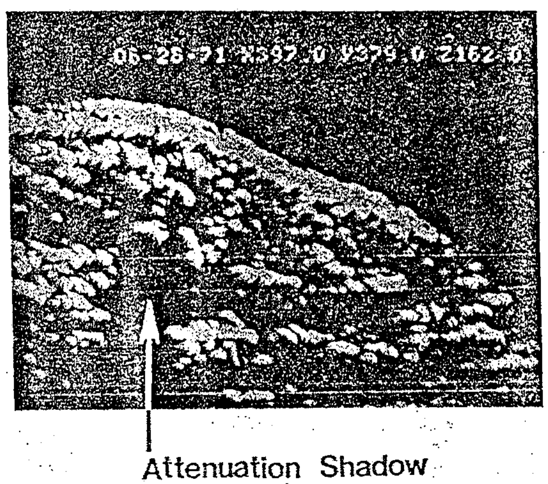


Fig. 7 Echogram obtained by longitudinal scan of formalin-fixed breast in region of malignant tumor. Fig. 8 shows a histological section of this breast specimen.

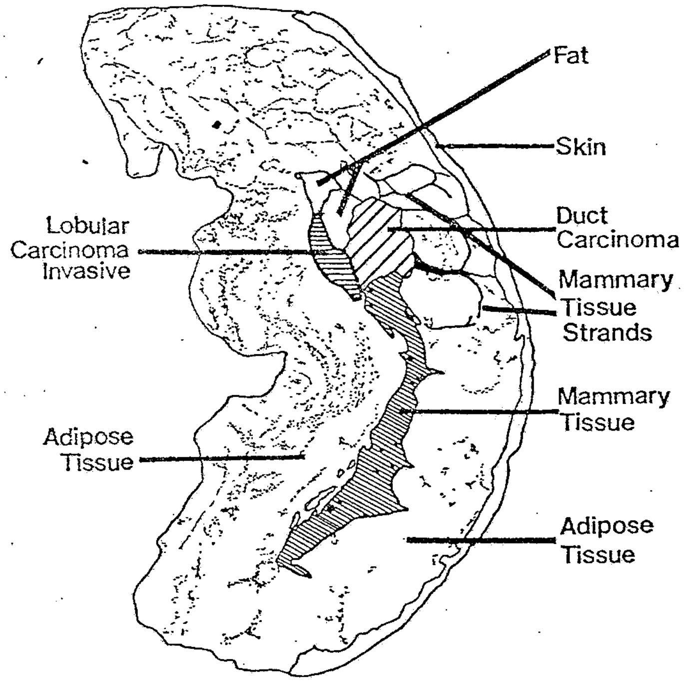


Fig. 8 Histological section of whole breast tissue with mixed type of malignant tumor. Echograms of this breast specimen shown in Figs. 7 and 9.

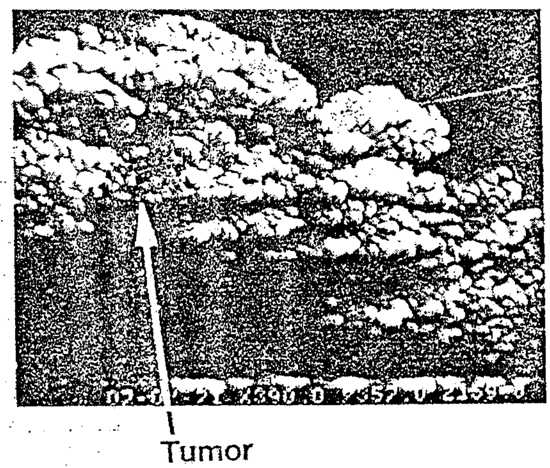


Fig. 9 Echogram obtained by longitudinal scan of formalin-fixed, in vitro breast with intact malignant tumor. Ductal cell carcinoma mass is imaged in region marked "tumor." Fig. 8 shows a histological section of this breast specimen.

It is commonly stated that 70 to 75% of breast carcinomas arise from ductal epithelium, 15 to 20% from lobular epithelium while the remainder are of undetermined source. This is probably an oversimplification. Recent observations strongly suggest that in many cases - perhaps as many as 50% - both cell types are present, duct cells usually in larger numbers. Neoplastic cells of ductal origin are comparatively large, variable in structure and differentiation and are arranged in nodules or form neoplastic ducts. About half of invasive masses containing predominantly duct cells are of the knobby pattern already described. Roughly a third are masses of stellate form, with long tentaculate projections extending outward in many directions. Such masses are characteristically diffusely fibrotic, the concentration of neoplastic cells varying little between the center and the periphery.

Invasive lobular carcinoma is typified by small cells with intensely hyperchromatic round or wedge shaped nuclei and scanty cytoplasm. They are arranged in rows a single cell wide between collagen fibers and form whorl-like formations around pre-existing normal ducts and blood vessels. Invasive masses thus formed are irregular in shape but lack the tentaculate projections of the stellate carcinoma of duct cell origin.

Figure 10 is a black and white duplication of one of the histological sections of a subject who had a large tumor categorized as invasive carcinoma in a region other than that shown in this figure.

The structure of the invasive carcinoma was somewhat unusual in that surrounding the main tumor mass and in all parts of the breast there was a spectacular involvement of intramammary lymphatic vessels by the carcinoma and from this intralymphatic tumor, innumerable tiny invasive nodules had arisen. Many of these were in remote parts of the breast. Many were only a fraction of a millimeter in diameter, while others were of the order of one millimeter or more in size. Many of these nodules were associated with the high density mammary tissue. Some of the invasive nodules are circled in Figure 10 in an attempt to differentiate them from other structures.

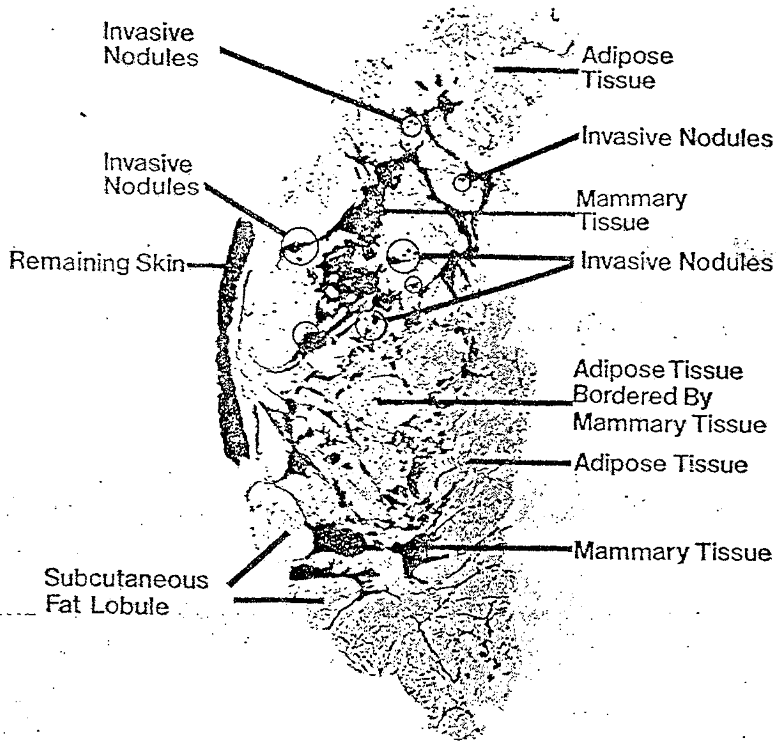


Fig. 10 Histological section of whole breast tissue with small malignant tumors.

The large primary tumor discussed above will not be discussed here but, rather, in order to illustrate the advantage of whole breast sectioning technique for checking the ability of ultrasound visualization systems to detect small tumors, only the results obtained for the minute, distributed tumors that were histologically identified in this specimen will be mentioned. The most significant result was that in examining the echograms (obtained with the 2.4 MHz transducer) prior to the histological sectioning, none of these small nodules were recognized. The smallest tumor mass detected with certainty was 0.5 cm in diameter. Other aspects of this will be discussed below.

5. DISCUSSION

Ultrasound visualization techniques can, at the present time, provide diagnostic information not otherwise available for some benign and malignant breast diseases. If such information is used in conjunction with the data provided by other methods, it contributes to successful differential diagnosis. However, as a result of the limited research on applications of ultrasound for breast examination, there is a lack of sufficient correlative data on the macro and micro structure of the various types of malignant breast tumors and the types of ultrasound imaging patterns they produce. If available, such correlative information could improve the present accuracy of differentiating between benign and malignant solid tumors and, in addition, should improve ultrasound visualization capabilities of detecting tumors less than 0.5 cm in diameter.

It is now generally agreed that some classes of malignant breast tumors attenuate ultrasound to a sufficient degree that they are easily detectable. Since benign tumors do not, apparently, significantly attenuate ultrasound, benign and malignant tumors can be differentiated by the "acoustic shadow" formed as a result of the attenuation.[20,45] The results obtained up to the present time may indicate that attenuation is characteristic of that broad classification of malignant tumors commonly referred to as "breast carcinoma" or "breast carcinoma of no special type." Included under this category are a wide range of histologic categories. All of the primary tumors discussed in this chapter come under this classification. If this prediction proves valid, it will be a significant factor in regard to the success of ultrasound as a diagnostic technique since the classification "breast carcinoma" represents approximately 75% of malignant breast tumors.

It deserves to be pointed out at this juncture that much of the confusion in published results of correlative studies is due to the chaotic nature of the classification of breast cancer among pathologists. The older classification systems, many still in widespread use, are empirically based and essentially illogical. Not only are different terms used for the same entity but, even more confusing, the same term may have entirely different significance in two different classification systems. The classification recently proposed by the Pathology Working Group of the National Cancer Institute's Breast Cancer Task Force[46] is the most orderly of the systems extant, but it has received little acceptance among pathologists.

To this difficulty is added the restriction of the sampling error inherent in conventional histologic procedures. When one realizes that a mass 3 cm in diameter has a volume in excess of 14,000 mm^3 and that a histologic section of average dimensions and thickness represents only a little more than 0.005% of this, the fallacy of extrapolation becomes apparent. As for the entire breast, assuming a volume on the order of 10^6 mm^3, even 100 average sections, a number far in excess of that usually prepared, would represent only 0.0001% of the total structure. It is predictable, therefore, that correlative studies employing conventional histologic sectioning and lacking close understanding between the representatives of the disciplines involved will produce only erratic and uninterpretable results.

Jellins, et al.[40] in ultrasound scans of *in vivo* breasts found the medullary tumor to be non-attenuating as did Calderon, et al.[47] in their *in vitro* studies of formalin-fixed breast tumors. Since not all malignant tumors attenuate the sound, and of the types that do exhibit this characteristic, the degree of attenuation is variable for the same frequency of sound, it is critically important to determine which parameters of the tumor structure are significant to the attenuation phenomena. It is equally important to determine which normal tissue structures will exhibit the attenuation characteristic. In this regard, it is of special interest that Kobayashi[48] found that fat necrosis within the breast is attenuating.

The results presented in this chapter are primarily concerned with the success of the experimental approach applied and the specific findings on overt tumors. The experiments, however, were multi-purposed in design and included, in addition to study of the malignant masses, the goal of the recognition, from the echogram pattern, of the normal structures within the breast. It should be realized in this regard that in ultrasound scanning of *in vivo* subjects and, in particular, middle-aged subjects who normally have large deposits of fat within the breast, as well as mammary tissue which is in a process of change, that the echograms obtained cover a rather wide range of differing patterns. Further, these patterns often include reflections from discrete mass structures that must be diagnosed as benign, malignant or normal. The information obtained on the appearance of normal structures within these specially prepared breasts were incorporated into the studies of *in vivo* breasts and is not discussed in this review of visualization of formalin-fixed preparations.

As indicated, the specific breast specimens that were studied in the experiments described here contained tumors that come under the general category mentioned above of carcinoma of the breast. None of these specimens contained significant calcium deposits (as revealed by x-ray examination) and no efforts were made to ultrasonically visualize calcium. The most significant finding, for the two frequencies used in this study (1.7 and 2.4 MHz), is that apparently the attenuation phenomena is associated with the total cellular mass, but dense necrotic-fibrotic tissue within this cellular mass increases the extent of the attenuation. It can be expected that the attenuation phenomena will be more pronounced with the higher frequencies.

The failure to detect the small distributed invasive tumors illustrated in Figure 8 is primarily associated with the frequency of the transducer and the necessary gain settings of the instrumentation. The 2.4 MHz unit was used in studying this particular specimen, and for physical target tests this focused transducer gives a range resolution of the order of one millimeter. Such resolution will not be realized at the gain settings required for visualization of the breast tissue structures. In re-examining the echograms, following the completion of the histological studies, some of the attenuating tumor masses under 0.5 cm in diameter could be recognized but this dubious level of recognition is not adequate.

The potential of the ultrasonic technique, in regard to specific display of the malignant mass as opposed to simply showing an acoustic shadow, even at the relatively low frequency (2.4 MHz) used in these early studies, is demonstrated in Figure 9. Clearly, comparative whole breast histology studies and ultrasound visualization will improve the present success in recognizing the differences between normal, benign and malignant structures. Further, it will stimulate changes in the design of transducers and associated instrumentation based on the knowledge of the precise structural features of breast tissue.

In considering the early results on these formalin-fixed specimens and data provided by subsequent studies of breasts of in vivo patients, one of the writers (E.K.F.) designed a simple, small diameter, 5 MHz, single focus transducer that is capable, when used in conjunction with adequate log amplifiers and display systems, of resolving some of the fine structural features within such excised, fixed breast preparations. Figures 11a and b are echograms obtained by linear scanning with the new transducer of a formalin-fixed breast specimen (obtained from M. D. Anderson Hospital) with an intact malignant tumor. At the time of this writing, only the ultrasound scanning has been completed. When the histological data are available, the results will be submitted for publication. The echograms are shown here only to demonstrate the level of improvement that can be obtained with transducers designed specifically for visualization of breast structures. In Figure 11a, which is a section through the approximate mid-region of the tumor (the location of the tumor was known from a radiograph of the specimen), sharp attenuation of the sound beam is evident; in Figure 11b, obtained by scanning one of the borders of the tumor, a distinctive outline of the tumor is obtained and the changes in the tissue in the narrow region extending from the tumor to the skin line can be recognized. Further, the various normal structures of the breast are displayed in excellent detail.

Finally, the correlation of whole breast histology and ultrasound vis-

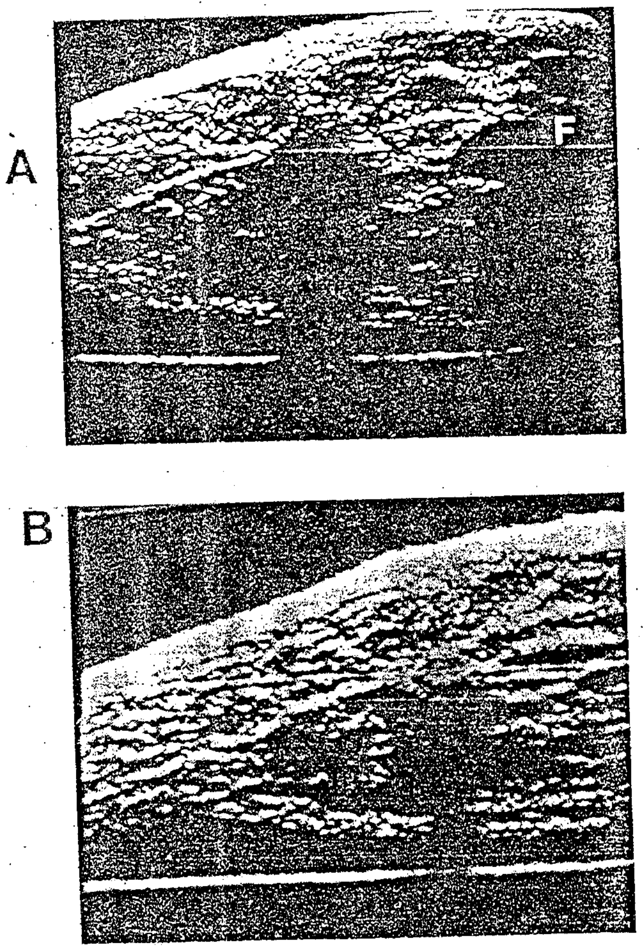


Fig. 11 A, B Echograms obtained by linear, longitudinal scans with 5 MHz focused transducer, of formalin-fixed, in vitro breast with intact malignant tumor. Echogram A represents mid-region of tumor; B, one border of the tumor.

ualization offers the opportunity to determine on a fundamental level the relation between frequency of the sound wave, echographic pattern and tissue structure. Earlier studies on liver demonstrated the differences in echogram pattern related to the varying connective tissue structure of the hepatic lobule of the liver of young pig and adult pig and other species.[32,49,50] It has been suggested that the elastic properties of tissues are largely responsible for the differences in echographic visibility by ultrasound techniques, and that the greatest difference in elasticity occurs at the interface of collagenous and non-collagenous tissue.[51] If valid, this is of fundamental significance to the visualization of pathological tissue masses which have altered collagen deposits. The Gallager and Martin[41,42] histological studies on classification of overt breast tumors have emphasized tissue configuration of the tumors such as cellular distribution, the diffuse or central character of fibrosis, collagen layers and necrosis. There is an immediate need for continued fundamental studies on the relation between the structure of the tumor mass, the echogram pattern and the dependence of this pattern on frequency of the applied ultrasound. However, there is also sufficient evidence on the generalized changes that take place in breasts containing a malignant tumor to encourage whole breast "histological-ultrasound visualization" studies aimed at detection of breast carcinoma prior to formation of an overt tumor mass. For example, in some breast carcinoma cases, Gallager and Martin[41,42] found changes in the supportive connective tissue, or an increased density of the breast due to proliferation of collagen in the mammary tissue and, in fact, consider these types of changes to be diagnostically significant. Insofar as early detection by ultrasound is concerned, the knowledge that such changes in the mammary tissue are not confined to the region of the tumor mass but are exhibited as widespread density effects should be a significant aid to pattern recognition of such changes. As shown in earlier publications, it is possible to recognize distributed changes within the breast.[27-31]

The above suggested "histological-ultrasound visualization" studies should, of course, include ultrasound visualization of the breasts of the patient prior to any surgical procedure, as well as study of the fresh and finally, the formalin-fixed breast. The relationship between early detection of breast carcinoma and the ultrasonic detection of specific alterations of ductal structures that sometimes accompany the malignant process has been discussed in a previous publication and will not be included here.[38]

6. SUMMARY

It was shown, as a result of the combined ultrasound and histological investigations, that: (1) ultrasonic scanning of formalin-fixed breast tissue is a valid and valuable research method, which can provide diagnostic information which is applicable to ultrasound scanning of the breasts of <u>in vivo</u> patients; (2) malignant masses classified under the broad category of "breast carcinoma" attenuated the incoming sound beam; (3) the attenuation of the sound was associated with the complete cellular malignant mass but was increased in regions of necrosis or fibrosis, (4) a breast carcinoma as wide as 3 cm in diameter was found to attenuate the sound beam, (5) it was possible, in some cases, to ultrasonically visualize the contours of the attenuating, malignant mass itself, and (6) some normal tissue configurations in the breasts of middle-aged subjects, such as a mass of fat surrounded by dense reflecting tissue, appeared on the echograms as encapsulated masses. Such normal tissues can be misdiagnosed as pathological tumors unless these patterns are recognized.

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