Phosphorus-31 magnetic resonance spectroscopic studies of malignant tissues have assessed phospholipid metabolism through the phospholipid precursors and products found in aqueous tissue extracts, namely phosphocholine and phosphorylethanolamine (Daly et al., 1987; Guidoni et al., 1987). These two principle precursor-products of phospholipid metabolism can be measured in analytical MR studies of perchloric acid extracts of human breast tissues (Merchant et al., 1988). These same two metabolites also constitute the majority of the signal detected in the phosphomonoester spectral region of intact cells and of in vivo MR analysed human breast tissues (Ronen et al., 1988; Sijens et al., 1988). Evaluated in this manner, these cytoplasmic components reveal little detail concerning the biochemistry of the corresponding membrane phospholipids in which they are incorporated.

Because phospholipids are a major component of the cell membrane, assessment of phospholipid metabolism in malignant tissues is important for understanding tumour growth (Rozengurt et al., 1979). Coman et al. (1944) showed that alterations in membrane metabolism and composition result in phenomena which lead to uncontrolled growth and loss of intracellular communication. Patton and Jensen (1975) showed that metastasis may be directly linked to alterations in cell membranes while other groups showed that phospholipids are important for the immune system’s response to tumour growth (Hefta et al., 1988; Low et al., 1988; Freddie et al., 1986).

Development of an analytical MR phospholipid analysis may be helpful in the diagnostic process. Such an analysis might, through characteristic differences in spectral profiles, enable differentiation of normal, benign, premalignant, and malignant breast tissues. This study profiles the phospholipids from normal, benign and malignant surgical breast tissue specimens with the intention of contributing to the understanding of the biochemistry of neoplastic processes in the human breast and advancing the role of magnetic resonance spectroscopy in differentiating malignant and benign lesions of the breast.

Materials and methods

Surgery

Human breast tissue specimens were obtained from patients undergoing scheduled surgical procedures. These unfixed tissue specimens were divided within 10 min into their diseased and nondiseased components for histopathologic examination and MR spectroscopic phospholipid analysis. Portions taken for MR analysis were then submerged in liquid nitrogen for storage. Specimens ranged from 0.2 to 1.5 g. The remaining surgical tissue specimens were sectioned and examined microscopically for histologic diagnosis after staining with hematoxylin and eosin.

Human breast tissue classification

Tissue specimens acquired for this study represent a variety of pathological conditions and were classified for purposes of univariate analysis as either malignant, benign or noninvolved breast parenchyma. Breakdown of these individual groups reveals 18 malignant specimens with a primary diagnosis of infiltrating adenocarcinoma; 25 benign specimens with primary diagnoses of benign conditions including fibrocystic disease and fibroadenoma; and 12 specimens of noninvolved breast parenchyma corresponding to ten noninvolved components of malignant and two corresponding noninvolved components of the benign specimens. The benign tissue specimens were further classified according to reported histology as fibrocystic disease and fibroadenoma for a separate multivariate analysis. The term ‘noninvolved breast parenchyma’ indicates that the tissue specimen was obtained beyond the margins of neoplastic tissues identified in the acquired breast tissue specimen. The term noninvolved breast parenchyma defines the essential and distinct tissue of the breast which is composed primarily of the glands and ducts. Note that specimens used in this study were from patients with no previous history of malignancy.

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Received 3 October 1990; and in revised form 2 January 1991.
Chemical procedures
A simple modified Folch extraction was used to extract the intracellular and membrane phospholipids (Folch et al., 1957; Meneses et al., 1988). Breast tissue specimens frozen in liquid nitrogen and placed in a fine powder using a stainless steel mortar and pestle chilled with liquid nitrogen. The homogenised tissue was added to 20 weight-volumes (1 gm = 1 ml) of chloroform-methanol 2:1 (v/v). The homogenate, having only one liquid phase, was filtered into a separatory funnel and washed with 0.2 volume of 0.1 M KCl and allowed to separate thoroughly (ca. 24 h) at 24°C. The chloroform phase, was recovered and evaporated at 37°C using a rotary evaporator. The analytical medium for the 31P magnetic resonance phosholipid analysis was identical to that previously described for MR phospholipid analysis (Meneses et al., 1988). The medium consists of reagent grade chloroform and methanol containing benzene-d6 and dissolved Cs-EDTA, pH 6.0. The prepared lipid that is free of excess solvents and not contaminated with excessive amounts of paramagnetic cations or free-radicals, was dissolved in 2.0 ml 2:1 chloroform-methanol containing benzene-d6 and placed in a 10 mm MR sample tube. A single phase was then obtained. To this solution, 1.0 ml of the Cs-EDTA salt was added and the mixture stirred. Two phases were obtained, a major chloroform phase and a minor water phase. The Teflon plug of the MR sample tube was advanced expulsing the minor water phase which was then removed. The MR sample tube turbine was adjusted so that only the chloroform phase was detected by the MR spectrometer’s receiver coil.

13P Magnetic resonance spectroscopy
The MR spectrometer used in this investigation was a heteronuclear GE 500NB system operating at 202.4 MHz for 31P. The spectrometer was equipped with an Oxford Instruments 500/52 magnet and cryostat, having an operating magnetic field of 11.75 Tesla, deuterium field-frequency stabilisation, and an automatic field-homogeneity adjustment capability that adjusted the spectrometer room temperature shims to improve field homogeneity during sample acquisition. Analytical samples were placed in standard 10 mm MR sample tubes and spun at 8 Hz during the data acquisition period. Samples were analysed with proton broad-band decoupling to eliminate 1H-31P MR multiplets. Under these conditions each spectral resonance corresponded to a single phosphorus functional group, representing a single generic phospholipid species. Chemical shift data are reported relative to the standard of 85% inorganic orthophosphoric acid (Glonek et al., 1974); however, the primary internal reference standard was a naturally occurring phospholipid phosphatidylcholine (chemical shift, −0.84 δ). Spectrometer conditions used for analytical extract analyses were as follows: pulse sequence, one pulse; pulse width, 18 μs (45° spin-flip angle); acquisition delay, 500 μs; cycling delay 500 μs; number of acquisitions, 12000; number of points per free-induction decay, 4096; acquisition time 1.02 s; sweep width ±1000 Hz. The total average time per analysis was 6 h. In addition, a computer generated filter time-constant introducing 0.6 Hz line broadening was applied. Data reductions, including peak area and chemical shift measurements and spectral curve analysis, were calculated using the spectrometer’s computer. To compensate for relaxation effects among various phosphorus signals detected in a single 31P MR spectroscopic profile, the MR spectrum must be standardised against measured amounts of tissue-profile metabolites wherever these are known. The rapid cycling time used necessitates calibration of the instrument with known materials appropriately doped to obtain calibrated spectra. The procedures for carrying out the calibration so that an accurate qualitative measurement is obtained from the 31P MR spectral profile have been described (Meneses et al., 1989; Greiner et al., 1981; Burt et al., 1976; Barany et al., 1982). Chemical shifts follow the convention of the International Union of Pure and Applied Chemistry and are reported in field independent units of δ.

Data analyses
Metabolite concentrations in relative phosphorus mole percentages were computed for all detected phospholipid resonances in the analysed breast tissue specimens. Mean metabolite concentrations for a relative phosphorus of phosphorus were calculated for the malignant, benign and noninvolved tissue groups. Initially, the three groups were compared at the level of the individual phospholipids by an analysis of variance. For those resonances where significance was determined to exist (F Probability, P < 0.05) post-hoc simple and complex contrasts were applied. Simple contrasts employed the Scheffé comparison procedure. Complex contrasts pitted the combined means of two tissue groups against the remaining mean to which a simple two-tailed t-test was applied (SPSS Inc., 1986). Significance was measured at the P < 0.05 and P < 0.01 levels for both post hoc comparison procedures. Under most conditions analysis of variance requires the assumption that the underlying variances between tested means are equal. At those resonances where significance was found to exist, homogeneity of variance was confirmed using Cochran’s C and the Bartlett-Box F tests.

From the grouped metabolite data, 19 indices representing phospholipid metabolism were calculated: PC + PE; PC plus PE plas; (PC plas + PE plas)/(PC + PE); PC plas/PE; LECITHIN, PC + PC plas; CHOLINE, PC + PC plas + SPH; LECITHIN/CEPHALIN, (PC + PC plas)/(PE + PE plas); OUTSIDE, PC + SPH; INSIDE, PE + PS; LEAF-LET, (PC + SPH)/(PE + PS); PC/PE, PC/PS, SPH/PS, PH/ PE; ANIONIC/NEUTRAL, (PC + PE)/(PC + PE plas); (PC + PC plas + SPH + PE + PE plas); LYSO, LPC + LPE; LPC/PC; LPE/PE. These theoretical parameters, given as ratios of individual or grouped phospholipids were generated to compare phospholipids or groups of phospholipids and provide more pathway-specific metabolic interrelations for discussion.

Benign breast tissue spectral data were analysed in a multivariate fashion. Using discrimination analysis, the variables (relative concentrations of LPC, U and PA) were entered in a forward, stepwise manner using P < 0.05 as criterion for inclusion in the model. Phospholipids independently significant in predicting benign tissue classification were determined.

For purposes of statistical analysis, missing values represent resonance signals lying below the levels of detection and were not included in the analysis.

31P MR phospholipid spectroscopic profiles of malignant, benign and noninvolved human breast tissues are presented in Figure 1. Thirteen resonance signals of the 31P spectra were identified as arising from membrane phospholipids. These resonance signals, from downfield, left to upfield right, include: PE at 0.52 δ, LPE at 0.43 δ, PA at 0.32 δ, CL at 0.18 δ, U at 0.13 δ, PE plas at 0.07 δ, PE at 0.03 δ, PS at −0.05 δ, SPH at −0.09 δ, LPC at −0.27 δ, PI at −0.37 δ, PC plas at −0.78 δ and PC at −0.84 δ.

The relative signal intensities of the noninvolved breast tissue phospholipid spectrum appear consistent in their arrangement with the most prominent signal, PC, followed successively in relative intensity by SPH, PE plas, PE, PI and other minor signals of variable intensity. This pattern is consistent in the benign spectra, but not in the malignant. In the malignant, the prominent PC peak is followed in relative intensity by a pattern of PE plas, SPH, and PE in which PE plas is most prominent of the three phospholipids in six cases, SPH most prominent in seven cases and PE most outstanding in five cases. PS is less prominent in all cases and is followed in relative intensity by PI.

Ten of the 12 noninvolved breast tissue specimens analysed were obtained from patients with diagnosed malignancy. Of these ten specimens, five demonstrated trace amounts of PA, LPC and U in various combinations, i.e. LPC appeared only
with PA and U present. PA did not appear in the remaining five specimens which also lacked detectable LPC and U. An appreciable characteristic of the noninvolved spectra was the absence of detectable LPE and PG.

In the benign spectra, the 25 specimens were derived from tissues with a primary diagnosis of fibrocystic disease in 13 cases and fibroadenoma in 12 cases. The pattern of concurrently appearing PA, LPC and the uncharacterised resonance at 0.13 δ was seen exclusively in the fibrocystic group and never in the fibroadenomas. Discrimination analysis using LPC, U and PA as independent features to predict fibrocystic disease vs fibroadenoma showed that LPC and U are independently significant. This model, a linear discrimination analysis, classified 92% of cases correctly by predicting the histologic tissue type. PA was significant in univariate analysis, however, when corrected for the contribution of LPC and U in the multivariate analysis, it was found not to be significant.

PA appeared in 14, LPC in 14 and the uncharacterised resonance at 0.13 δ in 16 of the 18 malignant cases studied. Other minor metabolites such as PG were also seen in these tissues.

Evaluation of the MR spectra of the three groups shows that quantitative spectral data (Table I) can be grouped into four ranges of relative concentrations: greater than 40%, PC; between 10 and 15%, PE plas and SPH; between 5 and 10%, PS and PE; and less than 5%, the eight remaining phospholipids. Among the remaining eight phospholipids, LPE was not detected in normal tissue and presented a relative mean concentration of less than 1% in malignant tissues. PG was detected only in one normal tissue sample and comprised less than 1% of neoplastic tissue phospholipids. A post-hoc Scheffé comparison procedure, applied between the means of the three tissue groups at the resonances of PE, LPC, PI and PC plas, following an analysis of variance, demonstrated significant differences among the three tissue groups (Table II, upper half). Malignant tissue is distinctly different from benign at PE, PI and PC plas, all three phospholipids are significantly elevated in malignant tissue by factors of 1.32, 1.33 and 1.25 respectively. Malignant tissue differed significantly from noninvolved at PE and LPC. PE was elevated by a factor of 1.22 and LPC was diminished by a factor of 0.44 in malignant tissue. Benign tissues differed significantly from noninvolved at LPC. They were diminished by a factor of 0.39 in benign.

Complex contrast of the individual phospholipids, comparing the combined mean values of two tissue groups to the remaining tissue group (Table III, upper half), showed that LPC and U were reduced and PE increased significantly in neoplastic tissue compared to noninvolved. SPH and LPC were significantly decreased in malignant tissue compared with nonmalignant while PE, PI and PC plas were significantly increased. Benign tissue differed significantly from the combined malignant and noninvolved with decreases noted in the resonances of PI and U.

To study patterns in the phospholipid metabolism of the three tissue groups, 19 indices were calculated from the spectra and compared with the histologic diagnoses of the tissue samples.
Table II  Significant simple contrasts of phospholipids and phospholipid indices using Scheffé comparison procedures

| Metabolite or Index | Malignant vs benign | Malignant vs noninvolved | Benign vs noninvolved |
|---------------------|---------------------|--------------------------|-----------------------|
| PE                  | *                   | *                        |                       |
| LPC                 | *                   | *                        |                       |
| PI                  | **                  | **                       |                       |
| PC plas had         | *                   | *                        |                       |
| PC plas/PC          | *                   | *                        |                       |
| PE plas/PE          | *                   | *                        |                       |
| CHOLINE             | *                   | *                        |                       |
| OUTSIDE             | **                  | **                       |                       |
| LPC/PC              |                     |                          |                       |

*PE, phosphatidyethanolamine; LPC, lysophosphatidylycholine; PI, phosphatidylinoisol; PC plas, phosphatidylcholine plasmanolagen; PC, phosphatidylcholine; PE plas, phosphatidylethanolamine plasmanolagen; CHOLINE, PC + PC plas + SPH (sphingomyelin); OUTSIDE, PC + SPH; LPC, lysophosphatidylcholine. *p < 0.05; **p < 0.01.

Table III  Post hoc tests performed as complex contrasts

| Phospholipid or Index | Noninvolved | Malignant vs combined |
|-----------------------|-------------|-----------------------|
| PE                    | *           |                       |
| SPH                   | *           |                       |
| LPC                   | *           |                       |
| PI                    | **          |                       |
| PC plas                | *           |                       |
| U                     |             |                       |
| PC plas/PC            | **          |                       |
| PE plas/PE            | *           |                       |
| CHOLINE               | **          |                       |
| OUTSIDE               | **          |                       |
| PC/PE                 | *           |                       |
| SPH/PE                | *           |                       |
| LYSO                  |             |                       |
| LPC/PC                |             |                       |

*PE, phosphatidyethanolamine; SPH, sphingomyelin; LPC, lysophosphatidylycholine; PI, phosphatidylinoisol; PC plas, phosphatidylcholine plasmanolagen; U, uncharacterised phospholipid; PC, phosphatidylcholine; PE plas, phosphatidylethanolamine plasmanolagen; CHOLINE, PC + PC plas + SPH; OUTSIDE, (PC + SPH)/PE; LYSO, LPC (lysophosphatidylcholine) + LPE (lyosphatidylethanolamine). *p < 0.05; **p < 0.01.

Table IV  31P MR phospholipid indices (± s.d.) of human breast tissues

| Phospholipid Index | Noninvolved | Benign | Malignant | F prob |
|--------------------|-------------|--------|-----------|---------|
| PC + PE            | 49.87 ± 3.96 | 51.52 ± 2.37 | 51.17 ± 4.67 | 0.56 |
| PC plas + PE plas  | 16.35 ± 3.49 | 15.55 ± 3.22 | 15.76 ± 3.07 | 0.78 |
| (PC + PE plas)/(PC + PE) | 0.32 ± 0.06 | 0.30 ± 0.05 | 0.31 ± 0.30 | 0.60 |
| PC plas/PC         | 0.09 ± 0.02 | 0.08 ± 0.01 | 0.11 ± 0.02 | 0.01 |
| PE plas/PE         | 1.65 ± 0.16 | 1.49 ± 0.19 | 1.18 ± 0.41 | 0.01 |
| LECTHIN            | 46.15 ± 3.60 | 47.07 ± 3.61 | 45.79 ± 3.73 | 0.50 |
| CHOLINE            | 59.51 ± 5.74 | 60.47 ± 5.68 | 56.26 ± 3.91 | 0.02 |
| LECTHIN/CEPHALIN   | 2.41 ± 0.78 | 2.46 ± 0.52 | 2.26 ± 0.38 | 0.60 |
| OUTSIDE            | 55.62 ± 4.27 | 56.72 ± 5.85 | 51.57 ± 3.96 | 0.005 |
| INSIDE             | 17.32 ± 6.16 | 16.94 ± 3.32 | 18.36 ± 2.58 | 0.26 |
| LEAFLET (PC + SPH)/(PE + PS) | 3.24 ± 0.48 | 3.55 ± 1.17 | 2.89 ± 0.69 | 0.07 |
| PC/PE              | 5.79 ± 1.43 | 5.62 ± 1.68 | 4.45 ± 1.79 | 0.04 |
| PC/PS              | 4.41 ± 0.65 | 5.58 ± 2.65 | 5.29 ± 1.91 | 0.30 |
| SPH/PS             | 1.41 ± 0.44 | 1.79 ± 1.25 | 1.29 ± 0.55 | 0.18 |
| SPH/PE             | 1.89 ± 0.82 | 1.88 ± 1.46 | 1.19 ± 0.73 | 0.12 |
| ANIONIC/NEUTRAL     | 0.22 ± 0.02 | 0.21 ± 0.02 | 0.04 ± 0.04 | 0.29 |
| LYSO               | 2.13 ± 0.54 | 1.53 ± 0.36 | 1.92 ± 0.32 | 0.13 |
| LPC/PC             | 0.05 ± 0.02 | 0.03 ± 0.01 | 0.03 ± 0.00 | 0.01 |
| LPE/PE             | 0.22 ± 0.19 | 0.06 ± 0.02 | 0.39 |

*PC, phosphatidylcholine; PE, phosphatidylethanolamine; PC plas, phosphatidylcholine plasmanolagen; PE plas, phosphatidylethanolamine plasmanolagen; LECTHIN, PC + PC plas; CHOLINE, PC + PC plas + SPH (sphingomyeline); LECTHIN/CEPHALIN, (PC + PC plas)/(PE + PE plas); OUTSIDE, PC + SPH; INSIDE, PE + PS (phosphatidylserine); LEAFLET, (PC + SPH)/(PE + PS); ANIONIC/NEUTRAL, (PI (phosphatidylinoisol) + PS + CL (cardiolipin) + PA (phosphatic acid) + PG (phosphatidylglycerol))/(PC + PC plas + SPH + PE + PE plas); LYSO, LPC (lyosphatidylethanolamine) + LPE (lyosphatidylethanolamine). *p = 12, 'p = 25, "p = 16. F probability of analysis of variance. *p < 0.01, *p < 0.05. 4Not detected in noninvolved tissues.

Discussion

The study of phospholipids in normal and pathological tissues is important because it can reveal membrane modifications produced by altered cellular conditions. Knowledge of these modifications is important for improvements in detection, diagnosis and techniques of intervention (Greig et al., 1986). This investigation utilises the techniques developed by Meneses and Glonek (1988) in their study of 31P MR spectra of extracted phospholipids. The unique quality of these techniques is derived from the fact that the complete phospholipid profile of a tissue, including phospholipids such as acyl-monoesters and diesters, can be separated by the procedure of Folch, Lees and Sloane-Stanley (1957). Further, the signal area in the MR spectrum represents the mole percentage of phosphorus concentration of all detectable phospholipids. Other methods such as thin-layer chromatography or high-performance liquid chromatography have none of these possibilities, since under the conditions of these procedures some signals may be masked by others and these procedures detect chemical characteristics such as double bonds or C = O functional groups rather than the phosphorus atom (Meneses et al., 1988) making them less reliable.
as a quantitative tool.

$^{31}$P MR phospholipid analysis permits the characterisation of the malignant, benign and noninvolved tissue types to the point that patterns in tissue spectra distinguish malignant from benign, malignant from noninvolved and benign from noninvolved (Figure 1). Observations made of differences in the order of relative intensities of phospholipids in the three tissue groups implies that differences exist among these tissue groups and that metabolism of phospholipids in the cancerous tissue is aberrant. It should be noted that the aberrations in the patterns of signal intensity described in the results hold true for the computed mean relative concentrations.

Fibrocystic disease

Given the definition of fibrocystic disease as a preneoplastic process by the American Academy of Pathologists (1986), the finding that LPC and U independently predicted the classifications of fibrocystic disease and fibroadenoma in 92% of the 25 benign cases may indicate that these resonances in combination are a spectral sign of premalignancy. The uncharacterised resonance at 0.13 δ has no statistical significance in simple comparison of means; however, it is not only independently significant in differentiating fibrocystic disease from fibroadenoma, it is significant in differentiating benign from combined malignant and noninvolved tissue and noninvolved from neoplastic tissue in complex contrasts. This phospholipid awaits isolation and further characterisation.

Phospholipid indices and complex contrasts

Phospholipid indices calculated in this study and presented in Table IV are designed to facilitate the interpretation of the spectral data presented in Table I in terms of biochemical pathways or interrelationships. The indices are practical for analysing different aspects of phospholipid metabolism. The plas/PE, for example, were only considered in those indices where their relationship with a regular phospholipid was established; therefore, the indices PC plas/PC and PE plas/PE are measures of the relationship of the more reduced enol-ether-containing plasmalogens to their more oxidised ester containing analogues in order to reflect the relative contribution of the metabolic pathways responsible for the biosynthesis of the ester-containing phosphatides and their corresponding enol ethers.

The use of complex contrasts in this analysis and in the general interpretation of MR spectral data is effective in that complex contrasts can define similarities and differences in processes or disease states of tissues. Not all of these differences and similarities can be explained in terms of known metabolic pathways or known alterations in membrane metabolism with disease. Indices are sometimes used to affirm or demonstrate trends in data. It should be noted that for the data presented in this study, when two complex contrasts are significant for a particular individual metabolite or index, the value of the noncombined group is less in one case and greater in the other. An example is PE, in which the noninvolved tissue level is significantly diminished compared to the neoplastic and the malignant is significantly elevated compared to the nonmalignant. The fact that a contrast cannot be interpreted in terms of a particular process should not detract from the value of contrast since it may represent processes unique to malignant or benign transformation.

Choline-containing phosphatides

Lysophosphatidylcholine, a product of phosphatidylcholine metabolism, is significantly decreased in both benign and malignant breast tissue. This is reflected in the complex contrast of normal and neoplastic tissues where the relative concentration of LPC in neoplastic tissues is depressed. Yamamoto and Ngwenya (1987) have reported that cancerous tissues release acyl-lysophospholipids as degradation products of acyl-phospholipoids, such as those containing choline. The reduction in the relative levels of LPC in the neoplastic tissues suggests that there is an increase in the turnover of this acyl-lysophospholipid compared to noninvolved tissues.

Phosphatidylcholine plasmalogens (an alkyl-phospholipid), was significantly elevated in the malignant tissues compared to benign. Snyder and Wood (1968) demonstrated that malignant tissues release alkyl-phospholipids as degradation products of cell membrane phospholipids. The elevation of acyllysophospholipid tissue to be elevated vs nonmalignant at this resonance was significant which further distinguishes the phenomena as an effect of malignancy.

The two major choline containing phospholipids, PC and SPH, studied as the index, OUTSIDE, represent the cell membrane’s major outer leaflet phospholipid components. The identity of PC and SPH as outer leaflet components in malignant tissues (Rothman et al., 1988) and nonmalignant cells (Rothman et al., 1977), and their position in the MR spectrum (Meneses et al., 1988) are well established. This index is diminished in the malignant tissue compared with the benign, which implies that the choline incorporated into the phospholipid is in the malignant tissue or that the choline is being utilised in other processes. This finding is further confirmed in the complex contrasts of this index which shows the index to be significantly diminished in malignant tissue compared to nonmalignant and to be significantly elevated in the benign tissue compared to the combined malignant and noninvolved. This alteration in membrane asymmetry, specifically the components of the outer leaflet, may be a result of the production of degradation or substitution products, i.e. lysophosphatidylcholine and PC plasmalogens.

Ethanolamine-containing phosphatides

The relative concentration of phosphatidylethanolamine is significantly enhanced in malignant tissue compared to benign and noninvolved. This elevation is confirmed in the complex contrast of malignant tissue against nonmalignant tissue which adds to a previous finding (Merchant et al., 1988) where the chemical residues of phosphatidylethanolamine, phosphorylethanolamine, glycerol 3-phosphorylethanolamine and α-glycerolphosphate were significantly elevated in malignant human breast tissues compared with benign and noninvolved tissues. This constellation implies that all the metabolites inphosphatidylethanolamine metabolism are elevated and is confirmed by the important role that PE has been found to play in the modification of membrane shape in malignant tissues (Cullis et al., 1985). The presence of trace amounts of LPE in neoplastic substances substantiates this conclusion.

The elevation of phosphatidylethanolamine and the reduction in choline-containing phosphatides SPH and PC, responsible for the bulk of membrane asymmetry in human tissues (Rothman et al., 1977), provides a basis for study of changes in membrane fluidity and noncompensatory changes in membrane phospholipid composition with disease.

Other phosphatides

Phosphatidylinositol was significantly elevated in malignant tissues compared with benign, but it did not show significant difference with the normal tissue in simple comparison as a consequence of the conservative statistical methods used. PI, however, can differentiate malignant tissue from nonmalignant and benign tissue from combined malignant and normal. The simple observation in the spectra that a difference is present in the relative spectral PI signal intensity can be attributed to the activation of protein kinase C pathway in malignant tissues since PI is one of the activators of this pathway (Turner et al., 1985; Price et al., 1989).

We conclude that qualitative and quantitative analysis of the $^{31}$P MR phospholipid profiles of human breast tissues utilising the described techniques is a worthwhile addition to
MR spectroscopic analysis of diseased human breast tissues. The value of MR spectral data has been demonstrated by predicting the benign tissue histology in 92% of cases.

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This research was supported by the intramural resources of the Pathology Institute, University of Utrecht and the Chicago College of Osteopathic Medicine.

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