

## Effects of the Anesthetic Dibucaine on the $^{31}\text{P}$ NMR Spectra of Maize Root Tissue

W. V. GERASIMOWICZ,\* M. J. KURANTZ, P. E. PFEFFER, and N. A. PSORSAS

*U.S. Department of Agriculture, Eastern Regional Research Center, 600 East Mermaid Lane, Philadelphia, Pennsylvania 19118*

We present the first *in vivo*  $^{31}\text{P}$  NMR study describing the effect of the anesthetic dibucaine on the levels of phosphorus-containing metabolites (G-6-P, P, NTP, NADP, and UDPG) in maize root tissue which was maintained in an aerobic state through modern perfusion techniques. The effects of dibucaine are demonstrated as a function of  $\text{Ca}^{2+}$  concentration at several pH values. Our results indicate that dibucaine alters the  $^{31}\text{P}$  spectra and the membrane-lipid composition of the roots upon introduction into the perfusate medium.  $\text{Ca}^{2+}$  inhibits the effects of the dibucaine as monitored by suppression of the  $^{31}\text{P}$  spectral changes induced by the anesthetic alone. Desaturase enzymatic inhibition has also been shown to occur in the presence of the anesthetic (in the absence of  $\text{Ca}^{2+}$ ), resulting in increased relative saturation of the fatty acids comprising the root tissue, with a marked decrease in linoleate (18:2) balanced by proportional increases in the more saturated fatty acid species. These compositional changes have been evaluated with respect to the real-time metabolic results as observed throughout the  $^{31}\text{P}$  NMR spectral studies.

Index Headings: Analytical methods; NMR; Spectroscopic techniques; Phosphorus;  $^{31}\text{P}$ ; *In vivo*; Dibucaine; Calcium.

### INTRODUCTION

The perturbations which anesthetics cause in animal membranes have been widely observed and studied.<sup>1,2</sup> Local anesthetics have been found to alter the permeability of phospholipid bilayer model membranes.<sup>3,4</sup> These species are also known to bind with isolated phospholipids while inhibiting cation binding,<sup>5-7</sup> and have been shown to be powerful inhibitors of cation binding to mitochondrial and submitochondrial particles.<sup>8</sup> The molecular mechanism whereby local anesthetics affect the membrane expansion and composition of plant cells and tissues is still not clearly delineated. In plant systems, anesthetics have been demonstrated to be effective in

overcoming dormancy and in stimulating germination of seeds through modification of membrane properties.<sup>9,10</sup> In particular, 2-butoxy-N-(2-diethylaminoethyl) cinchoninamide (dibucaine), a calmodulin antagonist, has been demonstrated to inhibit the rate of phosphatidylcholine hydrolysis in potato tuber homogenates.<sup>11</sup> This same anesthetic has also been shown to cause expansion of root membranes and resultant changes in their lipid composition.<sup>12</sup> Furthermore, dibucaine inhibits proton/cation translocation across the inner membrane of isolated corn mitochondria and prevents respiration-dependent contraction.<sup>13,14</sup>

Nuclear magnetic resonance (NMR) spectrometry allows us a unique opportunity to study the metabolite levels in maize root tips noninvasively, both in the presence and in the absence of dibucaine. <sup>31</sup>P NMR is now being utilized to monitor phosphorus-containing compounds such as glucose-6-phosphate (G-6-P), inorganic phosphate (P<sub>i</sub>), nucleotide triphosphates (NTP), nicotinamide adenine dinucleotide (NADP), and uridine diphosphate glucose (UDPG) in a variety of cell and tissue systems.<sup>15-22</sup> The levels of such metabolites are reflective of the energy status within *in vivo* systems. The chemical shift of the P<sub>i</sub> has also been used to determine intracellular pH.<sup>21,22</sup> No other single technique permits the monitoring of the various metabolites and their relative concentrations within unperturbed, intact, living cells. <sup>31</sup>P NMR was employed in this study to follow the intracellular phosphorous metabolite levels in maize root tissue upon exposure to dibucaine at several pHs, and as a function of calcium ion concentration at the respective pH values.

## MATERIALS AND METHODS

**Plant Materials.** Approximately 700–1000 corn root tips (3–5 mm in length) were excised from seedlings (*Zea mays* L. cv. FRB-73, Illinois Foundation Seeds†) germinated as previously described.<sup>23</sup> Immediately after cutting, the tips were placed into a cold neutral solution at 4°C and oxygenated for approximately 30 min. The plant tissue was then transferred to a 10-mm NMR tube (total sample volume, 2 mL) and immediately perfused at the rate of 45 mL/min in a slightly modified version of a perfusion system described previously.<sup>21,24</sup> O<sub>2</sub> was bubbled into the perfusate reservoirs throughout all of the experiments. The perfusates (1000 mL) consisted of (1) 10 mM morpholinopropane sulfonic acid (MOPS), pH = 7.5 (KOH adjusted), 50 mM glucose, and 0.0, 0.1, or 1.0 mM CaSO<sub>4</sub>; and (2) pH = 4.0, 50 mM glucose, and 0.0, 0.1, or 1.0 mM CaSO<sub>4</sub>, respectively. The solution pH values were monitored throughout all experiments and maintained (as necessary) with a few drops of dilute acid or base.

Dibucaine (0.05 mM) was added to the various perfusates after acquisition of a baseline <sup>31</sup>P NMR reference spectrum at the start of each experimental sequence.

**NMR Experiments.** All NMR data were obtained at 22 ± 1°C with a JEOL JNM-GX 400 FT NMR spec-

trometer. <sup>31</sup>P NMR spectra were acquired at 161.7 MHz. The resonance position of a 120 mM solution of hexamethylphosphoramide (HMPA)‡ in a sealed capillary tube centered in the sample tube was used as the external reference (assigned as 13.78 ppm) relative to methylenediphosphonic acid (MDP)<sup>25</sup> as a primary reference of 0.0 ppm. The <sup>31</sup>P NMR were obtained sequentially and stored on disk. Each spectrum was normalized in terms of S/N over the entire frequency range so that the relative concentrations of the components could be compared to the initial spectrum of each series. Each spectrum was signal averaged for 54 min. All spectral acquisitions were reproduced at least three times. Other parameters employed were: frequency range 16 kHz; 2 K data points zero-filled to 16 K; 20 K transients per spectrum; repetition time 162 ms; 12 μs (30° pulse); broadening factor 15 Hz.

**Lipid Analyses: Extraction and Quantitation.** Root tip samples were also treated on the bench, under conditions identical to the NMR experiments, in order to assess changes in lipid composition over the time course of the experiments. Untreated, freshly excised roots served as a control. The following samples were analyzed:

Untreated root tips—control

Root tips—pH = 7.5, 1.0 mM CaSO<sub>4</sub>, 0.05 mM dibucaine

Root tips—pH = 7.5, 0.05 mM dibucaine

Root tips—pH = 4.0, 1.0 mM CaSO<sub>4</sub>, 0.05 mM dibucaine

Root tips—pH = 4.0, 0.05 mM dibucaine

The treated root tips were exposed to the specified conditions (along with 50.0 mM glucose and O<sub>2</sub>) for the same lengths of times that were used for the NMR experiments. Treated and untreated root tip tissue was washed with deionized water (~100 mL/g fresh wt tissue) and drained by filtration on coarse scintered glass funnels. The tissue was immediately frozen in order to quench the possibility of any further reactions. We extracted the drained tissue two times, with 2:1 chloroform/methanol, by blending 50 mL of this mixture and filtering it through scintered glass. Extracts were dried by evaporation under nitrogen and treated as outlined by Folch *et al.*<sup>26</sup> Crude lipid obtained by this method was dried, weighed, and redissolved in chloroform. The chloroform extract was separated into neutral and polar fractions by TLC with the use of Analtech 500-μm prep plates and hexane: diethyl ether:acetic acid (70:30:1) as the solvent system.<sup>27</sup> Fatty acid methyl esters (FAMES) were prepared from each fraction by the method used by Slover and Lanza.<sup>28</sup> FAMES were analyzed by gas-liquid chromatography with a Hewlett-Packard 5992 instrument equipped with an FID detector and a 25-m OV101 fused-silica capillary column. Confirmation of peak identity was made with a Hewlett-Packard 5996 GC/MS.

## RESULTS

**<sup>31</sup>P NMR of Excised Maize Tissue Under Perfusion.** A <sup>31</sup>P NMR spectrum of the root tips obtained under aerobic conditions is shown in Fig. 1. The general characteristics and features of the spectrum agree with previously

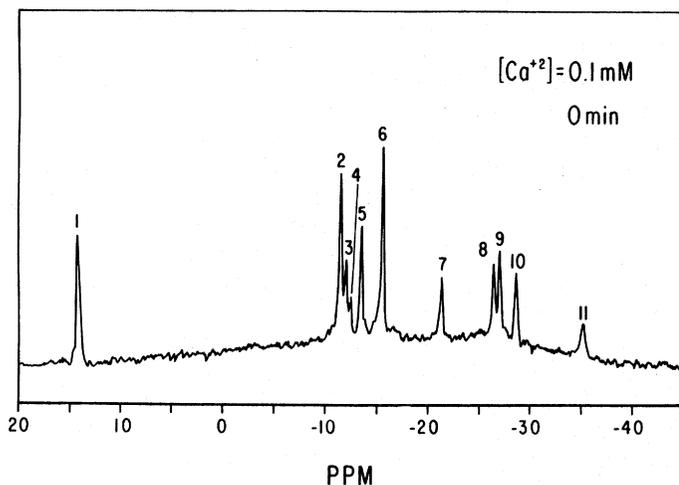


FIG. 1. The 161.7 MHz  $^{31}\text{P}$  NMR spectra of excised maize root tissue perfused with a solution comprised of 50.0 mM glucose, 0.1 mM  $\text{CaSO}_4$ , pH = 7.5, and  $\text{O}_2$  saturated. See Table I for  $^{31}\text{P}$  resonance assignments.

published information, and those  $^{31}\text{P}$  NMR resonance peak assignments were utilized in this work.<sup>18,20,21,24</sup> Table I contains a summary of the  $^{31}\text{P}$  chemical shifts and assignments of the peaks observed in the spectrum. The ratios of the nucleotide to non-nucleotide  $^{31}\text{P}$  peak heights and areas in the spectra presented in this paper are distorted, due to the rapid recycling times employed in acquiring the spectra (see NMR experimental section above). These distortions are due to differential longitudinal relaxation times among the various phosphorous metabolites, i.e., different  $T_1$  values. We have previously established these differences and have been able to account and correct for such effects in terms of response throughout this work and that previously described.<sup>21,29,30</sup>  $^{31}\text{P}$  NMR control spectra indicate that the basal stability of the root tissue can be maintained for periods of at least 23 h in perfusate medium free of dibucaine. Under such conditions, essentially no change in intracellular pH, intracellular compartmentation, membrane integrity, or total mobile phosphorus concentration is observed during the course of this lengthy time frame.

**Monitoring the Effects of Dibucaine *in vivo*.** Figures 2 and 3 depict  $^{31}\text{P}$  NMR spectra where the perfusate buffers were maintained at pH 7.5 and 4.0, respectively. The spectra shown in Figs. 2A, 2B, and 2C, and 3A, 3B, and 3C are representative of those acquired sequentially as a function of time, upon addition of 0.05 mM dibucaine to the perfusate medium in all cases, as well as a function of  $\text{Ca}^{2+}$  concentration. The first spectrum in each series (0 time) serves as a baseline reference point, in that no dibucaine is present throughout the acquisition. The times depicted in the sequential spectral plots represent the time intervals after dibucaine addition to the perfusate. The spectra shown in Fig. 2A and 2B indicate that the  $^{31}\text{P}$  metabolite levels remain stable even over very long periods, such as 1467 min after exposure to dibucaine. Although a small loss is apparent in the intensities of the sugar phosphate region, NTP and UDPG concentrations remain stable. Some broadening and a minor decrease in the cytoplasmic  $\text{P}_i$  intensity are also evident, in conjunction with an increase of mobile inorganic phosphorus in the vacuolar compartment of the

TABLE I. Overview of  $^{31}\text{P}$  NMR chemical shifts and assignments in maize.

Root tissue		
Peak	Chemical shift (ppm)	Assignments
1	13.78	HMPA <sup>a</sup>
2	-11.94	G-6-P
3	...	... <sup>b</sup>
4	...	... <sup>b</sup>
5	-13.96	$\text{P}_i$ cytoplasm
6	-16.00	$\text{P}_i$ vacuolar
7	-21.76	$\gamma$ -NTP
8	-26.76	$\alpha$ -NTP
9, 10	-27.31, 28.85	UDPG, NADP
11	-35.46	$\beta$ -NTP

<sup>a</sup> Chemical shift of HMPA, assigned a value of 13.78 ppm relative to MDP (0.0 ppm).

<sup>b</sup> Peaks 3 and 4 are thought to be fructose-6-P and AMP, respectively.

maize tissue. Figure 2C, however, shows a marked decrease in the  $^{31}\text{P}$  resonance intensities over much shorter time periods when compared to 2A and 2B. No calcium was present in the perfusate during this experiment. Losses in  $^{31}\text{P}$  signal intensity are apparent for all  $^{31}\text{P}$  NMR visible compounds, with a marked broadening of the signal corresponding to the inorganic phosphorus of the cytoplasm. Spectra taken at longer times show an almost complete loss of phosphorus signal, nonviability, and resultant metabolic death. Analogous experiments were performed at pH = 4.0, and selected spectra are depicted in Fig. 3. We note that, upon exposure to dibucaine, nucleotide and high-energy compound levels remain stable even when  $\text{Ca}^{2+}$  ions are not present in the system. Slight losses of G-6-P and other phosphorylated sugars are seen, along with some decrease of intensity and broadening of the cytoplasmic  $\text{P}_i$  resonance. However, in contrast to the data at the higher pH where  $\text{Ca}^{2+}$  is not present, the maize tissue is quite stable, even after 1380 min of exposure to dibucaine. The vacuole phosphate resonance increases in height as a function of time. Thus, total visible phosphorus actually increases, as seen in Fig. 5, where the concentration of  $\text{Ca}^{2+}$  ion is zero.

**Lipid Composition and Distribution.** Table II contains the data with regard to the amount of fresh weight tissue, crude lipid, and percentage of lipid material which could be extracted from the root tips after treatment with dibucaine under the various conditions. The effects of dibucaine on the membrane lipids of the maize root tips are depicted in Table III. This table lists the distribution of lipids (i.e., FAMES as detected by GC/MS) in both the neutral and polar lipid fractions. Dibucaine [2-butoxy-N-(2-diethylaminoethyl) cinchoninamide] has marked effects on the fatty acid distribution, as seen in neutral fraction 1 and polar fraction 6 of Table III. In both instances, the pH of the perfusate is at 7.5 and no  $\text{Ca}^{2+}$  ion is present. In sample 6, we note a large decrease in the proportion of linoleic (18:2) acid and a concomitant increase in the proportions of most of the saturated fatty acids (especially palmitic 16:0 and stearic 18:0), in comparison to samples 7 through 10. The degree of saturation, as reflected in the S/U ratio of Table III, is nearly threefold greater for sample 6, when compared with samples 7 through 10. These effects do not manifest themselves in the presence of the  $\text{Ca}^{2+}$  ion or at the lower pH, either in the presence or the absence of the  $\text{Ca}^{2+}$  ion.

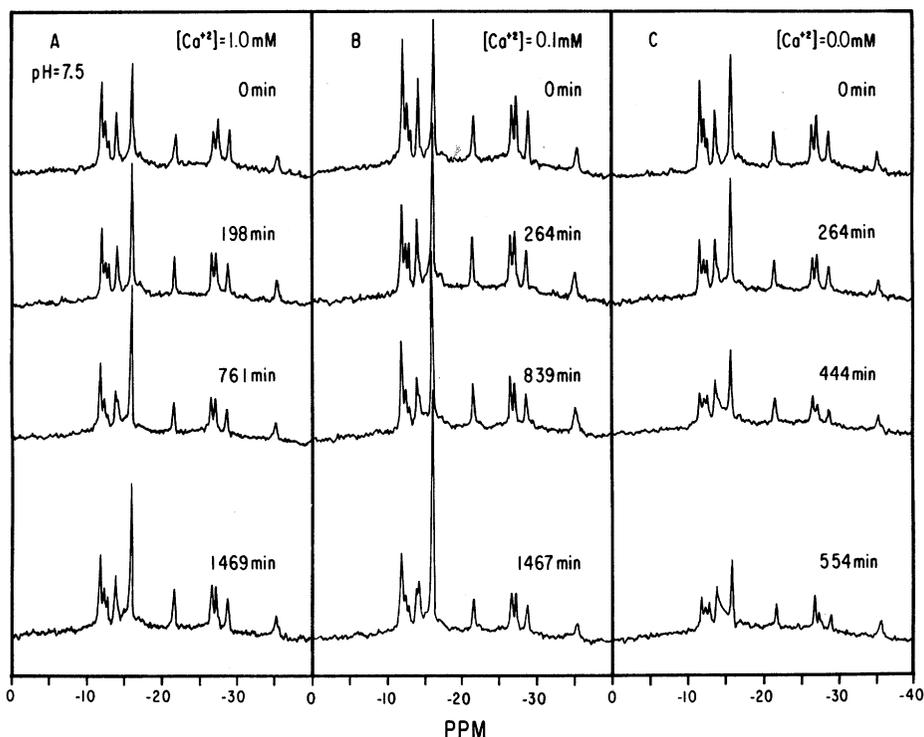


FIG. 2. (A, B, C)  $^{31}\text{P}$  NMR spectral responses of maize root tissue upon exposure to 0.05 mM dibucaine at pH = 7.5. (A) 1.0 mM  $\text{Ca}^{2+}$ ; (B) 0.1 mM  $\text{Ca}^{2+}$ ; (C) 0.0 mM  $\text{Ca}^{2+}$ .

Although the degree of saturation changes analogously for the neutral lipid (sample 1) fraction, the extent of the change in the S/U ratio of sample 1, compared with samples 2 through 5, is less than that seen for the polar lipid fraction (sample 6) when the analogous comparison

is made. The data in Table II illustrate the amount of lipid which could be extracted (after exposure to the anesthetic) from the maize tissue under various conditions. The effects of dibucaine on the distribution and composition of fatty acids appear to begin immediately

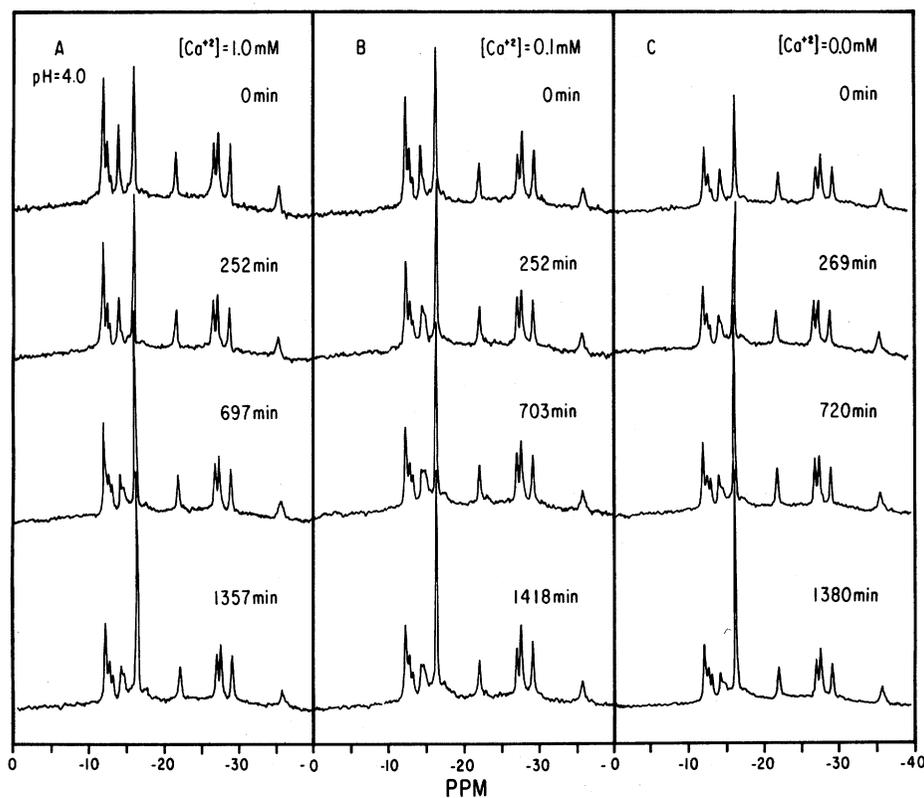


FIG. 3. (A, B, C) Same as Fig. 2, except that pH = 4.0.

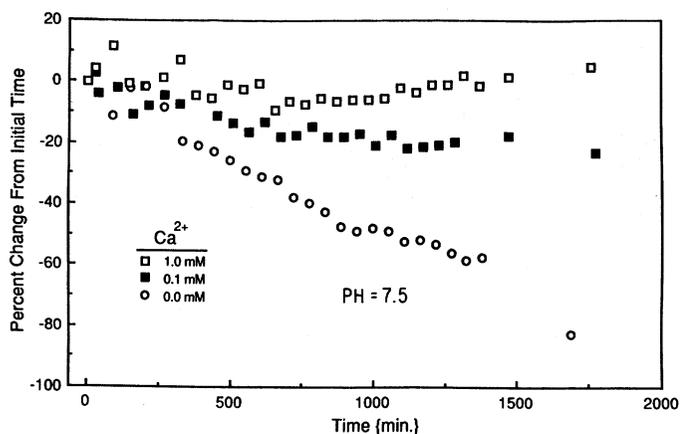


FIG. 4. Effect of dibucaine on total NMR-visible phosphorus ( $^{31}\text{P}$ ) at pH = 7.5.

upon exposure, as evidenced in both the analytical and spectroscopic studies.

## DISCUSSION

The unique combination of *in vivo*  $^{31}\text{P}$  NMR spectroscopy and lipid analysis demonstrates the ability to directly monitor the effects of dibucaine on the plant tissue examined in this report. The results are indicative of the fact that the action which dibucaine exerts on the root tissue is rapid, as observed in certain of the NMR spectra. Furthermore, such action is reflected in the perturbation of the composition of the polar and neutral lipid fractions, as well as in the loss of the total amount of fatty acid upon exposure to the anesthetic (Tables I and II). Dibucaine is known to cause expansion of root membranes, which in turn causes conformational and resultant compositional changes.<sup>12</sup> The potency of local anesthetics has also been correlated to their ability to interact with phospholipid model membranes and vesicles.<sup>3,5</sup> Such interactions of dibucaine with the lipid-protein portion of plant membranes should impart a greater fluidity to the membrane itself. As such, the plant appears to respond metabolically by producing saturated membrane fatty acids, which may stabilize or re-establish some rigidity to the membrane. Increases in saturation are apparent in Table III and are indicative of the inhibition or repression of physiological processes. The increase in saturation probably involves a reductive phenomenon which demonstrates desaturase inhibition.<sup>12</sup> More unsaturated lipid is present in the polar portions of root membrane systems, as seen in fractions 1 and 6 of Table III. As such, the dibucaine would be predicted to have a greater effect on the polar lipid fraction than on the neutral fraction. Our evidence clearly indicates that such is the case here (Table III). Since dibucaine is a calmodulin antagonist,<sup>11</sup> we see that  $\text{Ca}^{2+}$  (at sufficient levels) can block or inhibit the effectiveness of the anesthetic. The  $^{31}\text{P}$  NMR spectra further illustrate the competitive nature of cation binding vs. the binding of the anesthetic. In all cases (except at pH = 7.5 and 0.0 mM  $\text{Ca}^{2+}$ ) in Figs. 2 and 3 the phosphorus metabolite levels, particularly NTP, remain stable throughout the course of the experiment. A further example of the stability is illustrated in Figs. 4 and 5, where the total NMR-visible

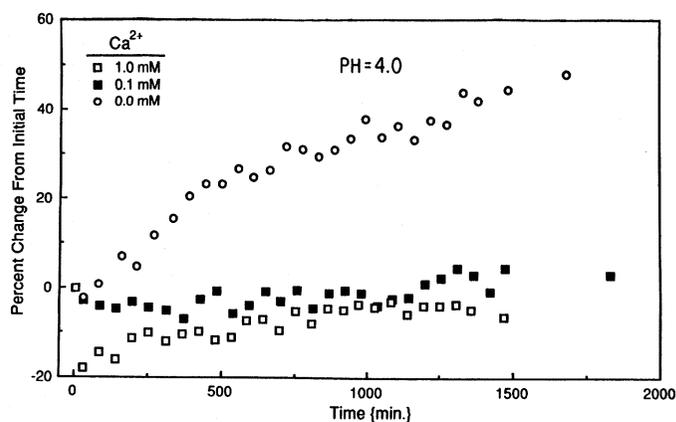


FIG. 5. Effect of dibucaine on total NMR-visible phosphorus ( $^{31}\text{P}$ ) at pH = 4.0.

$^{31}\text{P}$  is plotted over time. At  $\text{Ca}^{2+}$  levels of 0.1 mM and 1.0 mM (pH = 7.5 and 4.0), the percentage change of visible phosphorus is very low (0–10% at most), even over 20 to 24 h. At pH = 4.0 and 0.0 mM  $\text{Ca}^{2+}$  we actually see an overall increase in mobile phosphorus, mainly arising from vacuolar  $\text{P}_i$ . However, this increase has been previously reported and can be ascribed to the lower external pH condition as opposed to the action of the dibucaine.<sup>18</sup> When spectra (not shown) of the tissues which have not been exposed to either calcium or dibucaine are examined as controls at the two pH values, metabolite levels remain stable for periods of at least 18 h, with only very minor changes in mobile  $\text{P}_i$  or sugar and high-energy phosphorus levels. Only at pH = 7.5 and  $\text{Ca}^{2+} = 0.0$  mM do we see a drastic loss of total observable phosphorus (Fig. 4).

The lines of evidence elaborated above strongly suggest a correlation between the interactions of local anesthetics with acidic phospholipid membranes and the ability with which these species may displace  $\text{Ca}^{2+}$  from phospholipid headgroups. The chemical structure of dibucaine is such that it is a water-soluble organic amine which possesses appreciable lipid solubility. In physiological solutions around neutral pH, secondary and tertiary amino groups of anesthetics are partially in the ionized form due to their  $\text{pK}_A$  values. The  $\text{pK}_A$  of dibucaine is 8.5. Thus, both cationic and uncharged forms are present. The question regarding which form is the active compound is the subject of some debate.<sup>12</sup> Most

TABLE II. Extracted lipid and the effects of dibucaine.

Treatment	Data		
	Fr. wt tissue	Crude lipid (dry wt)	% Lipid
Untreated root tips	1.34 g	11.0 mg	0.82% (8.2 mg/g fr. wt)
Root tips DIB pH 7.5	1.15 g	8.8 mg	0.77% (7.7 mg/g fr. wt)
Root tips DIB pH 4.0	2.43 g	14.8 mg	0.61% (6.1 mg/g fr. wt)
Root tips Ca pH 4.0 DIB	2.94 g	14.1 mg	0.48% (4.8 mg/g fr. wt)
Root tips Ca pH 7.5 DIB	3.29 g	15.4 mg	0.47% (4.7 mg/g fr. wt)

TABLE III. Effects of dibucaine on membrane lipids of maize root tips.

Sample no. <sup>b,c</sup>	Fatty acid methyl esters (FAMES) area % <sup>a</sup>										Saturated/unsaturated
	14:0	15:0	16:0	17:0	18:2	18:0	18:3	20:0	22:0	24:0	
1	1.66	2.07	37.89	0.29	45.22	6.60	1.35	3.71	...	1.21	1.15
2	0.78	0.67	31.82	0.41	56.93	3.30	0.66	4.99	0.34	0.10	0.74
3	0.68	...	26.50	...	65.33	2.23	...	1.74	...	3.52	0.53
4	0.68	0.65	29.27	...	61.50	2.44	0.34	2.60	...	2.52	0.62
5	0.83	1.22	23.87	...	61.34	8.21	0.47	3.18	...	0.88	0.62
6	0.72	1.93	48.88	...	36.92	4.63	2.82	2.85	...	1.38	1.52
7	...	...	30.73	...	62.63	2.15	0.76	2.01	1.10	0.62	0.58
8	...	...	29.50	...	65.33	2.76	0.52	1.89	...	...	0.52
9	0.21	0.44	29.74	...	64.46	2.70	0.67	1.78	...	...	0.54
10	...	...	29.77	...	62.93	5.49	...	1.81	...	...	0.59

<sup>a</sup> The uncertainties in the areas are approximately  $\pm 5\%$  of the actual reported values based upon three replicates in all cases.

<sup>b</sup> 1. Neutral Fr. DIB pH 7.5

5. Neutral Fr. N/T

8. Polar Fr. DIB pH 4.0

2. Neutral Fr. DIB, Ca pH 7.5

6. Polar Fr. DIB pH 7.5

9. Polar Fr. DIB, Ca pH 4.0

3. Neutral Fr. DIB pH 4.0

7. Polar Fr. DIB, Ca pH 7.5

10. Polar Fr. N/T

4. Neutral Fr. DIB, Ca pH 4.0

(N/T = untreated samples)

<sup>c</sup> FAMES which were not detected at all are not listed (e.g., 18:1).

<sup>d</sup> (···) Not detected.

recent evidence appears to be in favor of the cationic form as the active species.<sup>12</sup> However, the <sup>31</sup>P spectral and lipid analytical data intimate that the dibucaine is more effective at the more alkaline pH. This relatively higher potency has been explained on the basis of increased lipid solubility for the uncharged form.<sup>31,32</sup> This property enables the uncharged species to penetrate cellular diffusion barriers under alkaline conditions. After traversing such barriers, local dibucaine molecules would reach areas adjacent to the plasmalemma, where they are presumably effectively buffered in living plant cells, and would therefore be converted back to the cationic state. Figure 2C reveals that the cytoplasmic P<sub>i</sub> peak broadens as a function of time as the dibucaine interacts. This broadening indicates that the pH is becoming somewhat more acidic in portions of the cytoplasmic compartment,<sup>21,22</sup> and it would be consistent with conversion of the anesthetic through such buffering action to the cationic form.

The effects of dibucaine, as manifested analytically and now spectroscopically, may be the result of any one of a number of factors influencing cellular metabolism individually and/or in concert. Anesthetics, such as dibucaine, appear to induce changes which closely resemble those produced by organic acids and undissociated phenolic compounds.<sup>12</sup> In all cases, the effects of the anesthetic are seen through hydrogen bonding, and thus protonation of the substrate. Dibucaine has been reported to inhibit the formation and maintenance of proton gradients, i.e., to cause the dissipation of  $\Delta$ pH gradients.<sup>25</sup> ATP-mediated H<sup>+</sup>-translocations and Ca<sup>2+</sup> ion transport across membrane vesicles are also inhibited by dibucaine.<sup>25</sup> Furthermore, dibucaine inhibits membrane-bound ATPase activity.<sup>25</sup>

In corn mitochondria, dibucaine has been reported to inhibit both proton transport facilitated by the ATPase complex and protonophore-stimulated respiration.<sup>14</sup> Dibucaine may interact with membrane phospholipid and regions of membrane protein through a combination of hydrophobic and electrostatic effects. The electrostatic effects result in a reduction of local charge density and prevent cation binding and subsequent transport of cat-

ions (including protons) across membranes.<sup>8</sup> These phenomena are reflected in the acidification of the cytoplasmic P<sub>i</sub> resonances and in the general loss of overall <sup>31</sup>P NMR-visible signal seen in the spectra of Fig. 2C. The uniform reduction of signal seen in this figure may indicate general leakage of the phosphorus from the cells or the breakdown of cellular membrane integrity. At pH = 4.0 the membranes and <sup>31</sup>P spectra appear to remain intact, whereas at pH = 7.5 (dibucaine and no Ca<sup>2+</sup>) membrane disruption appears to be evident, along with gross FAME distributional changes. Although Ca<sup>2+</sup> appears to inhibit the effects of dibucaine on the disruption of cellular integrity, the presence of this ion does not provide a means of totally preventing changes in the membrane composition (Table III) upon exposure of the maize tissue to the substrate, even in the presence of calcium ion.

The next level of understanding will be achieved as we begin to focus upon the individual factors responsible for the effects described. Certainly, spectroscopic emphasis on the examination of other nuclei and on the fate of the substrate itself, upon incorporation into the maize tissue, will potentially extend our current findings.

The effects of dibucaine, and therefore other anesthetics and organic acids, can be studied by <sup>31</sup>P NMR for extended time periods under various conditions of pH and Ca<sup>2+</sup> ion concentration. Furthermore, the interaction of dibucaine with phospholipid membranous tissue is antagonized by Ca<sup>2+</sup> (i.e., inhibited). Further work with other anesthetics and metal ions will serve to enhance our understanding of the molecular mechanisms underlying physiological processes in whole plants and tissues, in that correlations between extracts and actual *in vivo* systems are not always accurate.

1. *Molecular Mechanisms of Anesthesia*, B. R. Fink, Ed. (Raven Press, New York, 1975).
2. C. D. Richards, "Anesthetics and Membranes," in *Biochemistry of Cell Walls and Membranes*, J. C. Metcalf, Ed. (University Park Press, Baltimore, 1978), pp. 157-220.
3. D. Papahadjopoulos, *Biochim. Biophys. Acta* **265**, 169 (1972).
4. M. A. Singer and K. J. Mahendra, *Can. J. Biochem.* **58**, 815 (1980).
5. M. B. Feinstein, *J. Gen. Physiol.* **48**, 357 (1964).

6. M. B. Feinstein and M. Paimre, *Biochim. Biophys. Acta* **115**, 33 (1966).
7. E. Rojas and J. M. Tobias, *Biochim. Biophys. Acta* **94**, 394 (1965).
8. A. Scarpa and A. Azzi, *Biochim. Biophys. Acta* **150**, 473 (1968).
9. S. B. Hendricks and R. B. Taylorson, *Planta* **149**, 108 (1980).
10. R. B. Taylorson and S. B. Hendricks, *Planta* **145**, 507 (1979).
11. R. A. Moreau, T. F. Islett, and G. J. Piazza, *Phytochemistry* **24**, 2255 (1985).
12. P. C. Jackson and J. B. St. John, *Planta* **162**, 415 (1984).
13. R. P. Klein and D. E. Koeppe, *Plant Physiol. (Bethesda)* **67** (4 Suppl.) 9 (1981).
14. R. P. Klein and D. E. Koeppe, *Plant Physiol.* **77**, 99 (1985).
15. P. Brodelius and H. J. Vogel, *Enzyme Engineering* **7** **434**, 496 (1984).
16. C. T. Burt, *Phosphorus NMR in Biology* (CRC Press, Boca Raton, Florida, 1986).
17. D. G. Gadian, *NMR and Its Applications to Living Systems* (Oxford University Press, Oxford, 1982).
18. W. V. Gerasimowicz, S. Tu, and P. E. Pfeffer, *Plant Physiol.* **81**, 925 (1986).
19. D. G. Gorenstein, *<sup>31</sup>P NMR: Principles and Applications* (Academic Press, New York/London, 1984).
20. P. C. Jackson, P. E. Pfeffer, and W. V. Gerasimowicz, *Plant Physiol.* **81**, 1130 (1986).
21. P. E. Pfeffer, S. Tu, W. V. Gerasimowicz, and J. R. Cavanaugh, *Plant Physiol.* **80**, 77 (1986).
22. J. K. M. Roberts, P. M. Ray, N. Wade-Jardetzky, and O. Jardetzky, *Nature* **283**, 870 (1968).
23. S. Tu, G. Nagahashi, and B. J. Sliwinski, *Biochem. Biophys. Res. Commun.* **122**, 1367 (1984).
24. R. B. Lee and R. G. Ratcliffe, *J. Exp. Bot.* **34**, 1213 (1983).
25. N. Agarawal and V. K. Kalra, *Biochim. Biophys. Acta* **764**, 316 (1984).
26. J. Folch, M. Lees, and G. H. Sloane-Stanley, *J. Biol. Chem.* **226**, 497 (1957).
27. H. B. White and S. S. Powell, *Biochim. Biophys. Acta* **116**, 388 (1966).
28. H. T. Slover and E. Lanza, *Journal of the American Oil Chemists' Society* **56**, 933 (1979).
29. P. E. Pfeffer, S. Tu, W. V. Gerasimowicz, and R. T. Boswell, *Planta* (In Press) 1987.
30. T. Schleich, J. A. Willis, and G. B. Matson, *Exp. Eye Res.* **39**, 455 (1984).
31. E. J. Ariens and A. M. Simonis, *Arch. Int. Pharmacodyn.* **141**, 309 (1963).
32. J. M. Ritchie and B. R. Ritchie, *Science* **162**, 1394 (1968).