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PART I

FIELD DESORPTION MASS SPECTROMETRY OF PHOSPHOLIPIDS

PART II

CHARACTERIZATION OF MODIFIED NUCLEOSIDES AND NUCLEOTIDES BY FIELD DESORPTION MASS SPECTROMETRY

by

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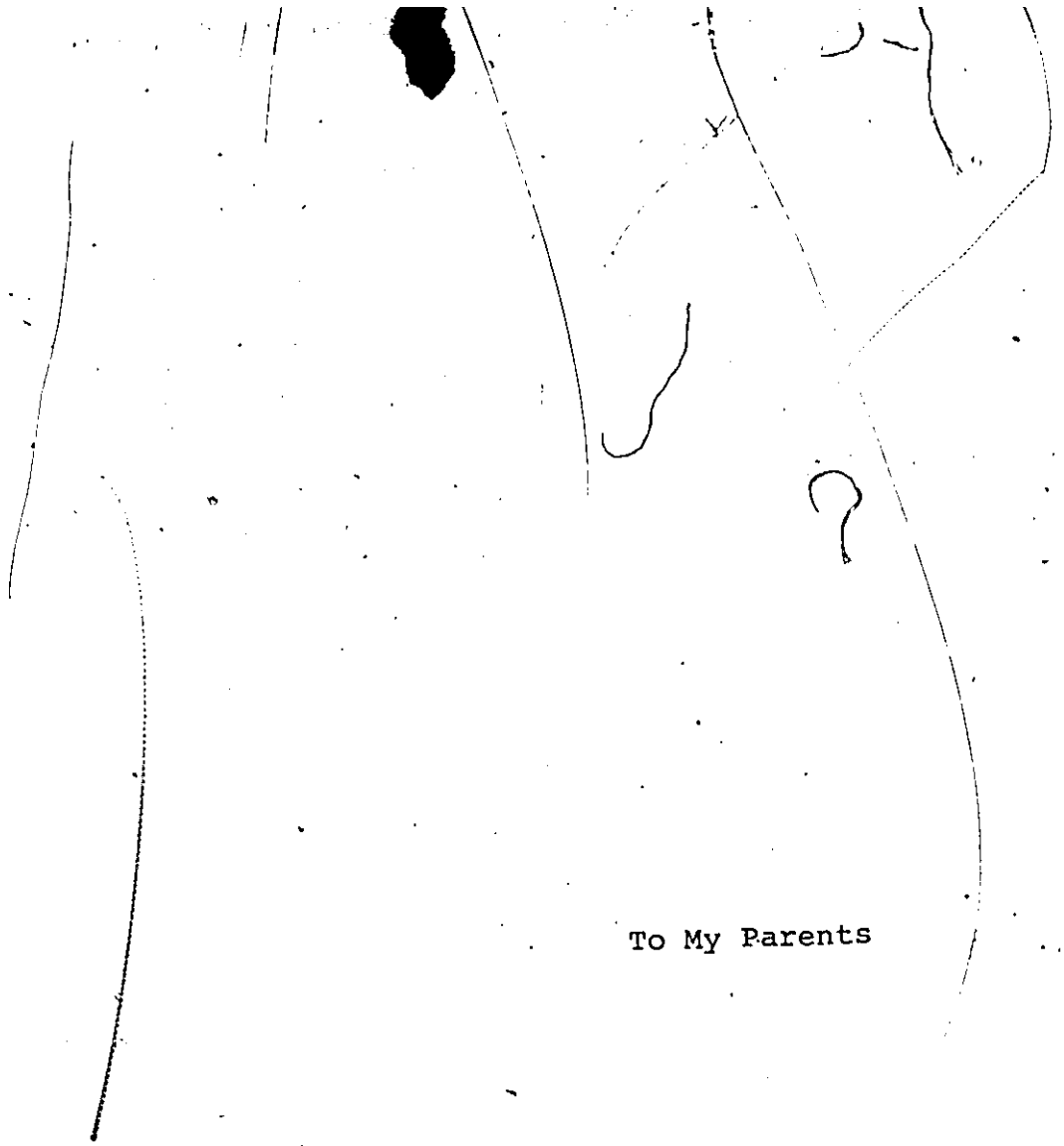

To My Parents

ABSTRACT

PART I

Field desorption mass spectra of 17 synthetic PCs, 5 LPCs, 4 PEs, 2 LPEs, 1 PDME, 2PAs, 3 LPAs, 1 PG, 2 phosphoryl amino alcohols, 3 glycerophosphoryl amino alcohols, 3 sphingolipids were studied. Most spectra display the $[M+H]^+$ protonated molecular ion as the base peak but they generally also contain significant fragments. Some characteristic features of f.d.m.s. such as fragmentation of protonated molecular ions by simple bond cleavage, elimination of neutral particles, formation of field-/ thermally-induced associated ion clusters and hydrates, field-induced multistep rearrangement and phosphorylation, and the occurrence of doubly charged ions appear in a number of phospholipids. All these processes are strongly influenced by the anode heating current. Formation schemes of the major ions are proposed and discussed.

PART II

The feasibility of applying f.d.m.s. in identification of nucleic acid components in DNA hydrolysates is demonstrated. The f.d. mass spectra of 6 methylated pyrimidine and purine bases, 24 normal and methylated nucleosides and 6 nucleotides are presented. With carefully

controlled anode heating, most of the compounds yield an $M^{+\cdot}$ or $[M+H]^+$, and the characteristic sugar (S) and base (B+H) cleavage products are the major fragments. An equimolar mixture of 5 authentic major nucleosides as well as 3 fractions of a DNA hydrolysate were also analyzed.

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PART II

TABLE OF ABBREVIATIONS

a.m.u.	atomic mass unit
B	base, molecular weight of the free base-1
BAT	best anode temperature (current) or optimal anode heating current
cf.	compare
c.i.	chemical ionization
c.i.m.s.	chemical ionization mass spectrometry
DNA	deoxyribonucleic acid
ECP	emitter current programmer
e.g.	for example
e.i.	electron impact
e.i.m.s.	electron impact mass spectrometry
eV	electron volts
f.d.	field desorption
f.d.m.s.	field desorption mass spectrometry field desorption mass spectrometer field desorption mass spectrum (or spectra)
f.i.	field ionization
f.i.m.s.	field ionization mass spectrometry
g.c.m.s.	gas-liquid chromatography and mass spectrometry combination
g.l.c.	gas-liquid chromatography
HMD	hyaline membrane disease
i.e.	that is
int.	intensity
i.p.	ionization potential

TABLE OF ABBREVIATIONS (cont'd)

i.r.	infared
L	lecithin
LPA	lysophosphatidic acid
LPC	lysophosphatidyl choline
LPE	lysophosphatidyl ethanolamine
m/e	mass to charge ratio
m.s.	mass spectrometer/spectrometry
mol. wt. (M.W.)	molecular weight
N	ribonucleoside acid
dN	2'-deoxyribonucleoside
NDP	ribonucleoside diphosphate
dNDP	2'-deoxyribonucleoside diphosphate
NMP	ribonucleoside monophosphate
dNMP	2'-deoxyribonucleoside monophosphate
NTP	ribonucleoside triphosphate
dNTP	2'-deoxyribonucleoside triphosphate
PA	phosphatidic acid
PC	phosphatidyl choline
PDME	phosphatidyl N,N-dimethylethanolamine
PE	phosphatidyl ethanolamine
PFK	perfluorokerosene
PG	phosphatidyl glycerol
PI	phosphatidyl inositol
PS	phosphatidyl serine
phC	phosphoryl choline
phE	phosphoryl ethanolamine

RDS	respiratory distress syndrome
rel. int.	relative intensity
RNA	2'-ribonucleic acid
S (in PART I)	sphingomyelin
S (in PART II)	sugar
SEM	secondary electron multiplier
S_N	nucleophilic substitution
t.l.c.	thin layer chromatography
TMS	trimethylsilyl
TRIS	tris-(hydroxymethyl) aminomethane
μm	micrometer

FREE BASES

Ade	adenine
Cyt	cytosine
Gua	guanine
Thy	thymine
Ura	uracil
1-MeAde	1-methyladenine
2-MeAde	2-methyladenine
3-MeCyt	3-methylcytosine
7-MeGua	7-methylguanine
1-MeUra	1-methyluracil
6-MeUra	6-methyluracil

TABLE OF ABBREVIATIONS (cont'd)

NUCLEOSIDES

A	adenosine
dA	2'-deoxyadenosine
C	cytidine
dC	2'-deoxycytidine
G	guanosine
dG	2'-deoxyguanosine
T	thymidine
U	uridine
dU	2'-deoxyuridine
1-MeA	1-methyladenosine
N^6-MeA	N^6-methyladenosine
1-MedA.HCl	1-methyl-2'-deoxyadenosine hydrochloride
N^6-MedA	N^6-methyl-2'-deoxyadenosine
3-MeC	3-methylcytidine
5-MeC	5-methylcytidine
1-MeG	1-methylguanosine
7-MeG	7-methylguanosine
N^2-MeG	N^2-methylguanosine
1-MedG	1-methyl-2'-deoxyguanosine
3-MeT	3-methylthymidine
3-MeU	3-methyluridine
5-MeU	5-methyluridine

TABLE OF ABBREVIATIONS (cont'd)

5-HmdU	5-hydroxymethyl-2'-deoxyuridine

NUCLEOTIDES

3'(2')-AMP	3'(2')-adenosine-5'-monophosphosate
5'-AMP	adenosine-5'-monophosphate
5'-CMP	cytidine-5'-monophosphate
5'-dCMP	deoxycytidine-5'-monophosphate
5'-GMP	guanosine-5'-monophosphate
5'-TMP	thymidine-5'-monophosphate

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PART I

FIELD DESORPTION MASS SPECTROMETRY OF PHOSPHOLIPIDS

CHAPTER I

INTRODUCTION

This dissertation describes the application of a new mass spectral technique to structure determination and analysis of intact phospholipids. In this section, the following aspects will be discussed:

1) biomedical significance of phospholipids,
2) analytical methods in phospholipid analysis,
3) mass spectrometric analysis of phospholipids,
4) application of field desorption mass spectrometry (f.d.m.s.) to intact phospholipid analysis.

The fetal lung undergoes extensive anatomic and histologic changes during gestation in preparation for its role as an organ for gas exchange in the neonate.[1] To successfully adapt to the air breathing state, the fetal lung must also develop the capacity to produce surface active phospholipids (phosphatidyl cholines (PC), commonly called lecithin, are thought to be the critical components[2]) in the aveolar lining layer.[3,4] This layer reduces surface tension at the air-tissue interface, preventing the collapse of alveoli on expiration.[5,6] Maturation of the lung, at about 35 weeks gestation, is associated with a big increase in lecithin (particularly dipalmitoyl phosphatidyl choline[2,7,8]) synthesis and a corresponding rise in concentration of this class of compounds in amniotic fluid.[9,10,11] A deficiency

of lecithin has been shown to be the leading cause of the breathing problems of the premature infants developing respiratory distress syndrome (RDS) and subsequent hyaline membrane disease (HMD)[12-14]. Determination of amniotic fluid phospholipids[15-21] has proved a useful guide to the prenatal assessment of fetal lung maturity such that the delivery may be induced or postponed accordingly.

Most of the methods used so far for following the change in lecithin concentration have employed thin-layer chromatography (t.l.c.) of a lipid extract of the amniotic fluid. In general, the lecithin (L) spot on the chromatogram after staining is compared with that of sphingomyelin (S), the L/S ratio is then assessed visually or densitometrically.[22-30] Alternatively, the lecithin concentration can be estimated either by staining with a reagent for phosphate detection[31-33], or by scraping the material off the plate and determining the phosphate content[34,35].

However, the interpretation of L/S ratio is open to some questions[15,36,37]. For example, the densitometric measurement is only semiquantitative and the results can be affected by differences in spot sizes, staining techniques, variation in R_f values, etc. Furthermore, the L/S ratio may be a measurement of fetal age rather than lung maturation[18]. Determination of lecithin phosphate after removal from the TLC plate may offer some advantages but it is extremely time

consuming and probably dependent on the amniotic fluid volume[38] as well.

Recently, a different approach to this problem has been developed[39-41] by measuring the total palmitic acid in amniotic fluid by means of gas-liquid chromatography (g.l.c.). Although most of the neutral lipids were removed successfully and the palmitic acid was reported to come mostly from lecithin[36], it is difficult to ensure that none of the palmitic acid residue is derived from other phospholipids.

In addition, chromatographic procedures may be used to separate the various classes of phospholipids[42-44]. It has been shown that the separation of individual phospholipids within a class is rather cumbersome and it is a demanding technique that requires considerable experience. Various chromatographic techniques[45] including argentation t.l.c. (adsorption or reverse phase)[46-48]; reverse-phase partition chromatography[49,50]; mercuric acetate adduction of unsaturated centres; the latter followed by adsorption or partition chromatography[51], 2-dimensional t.l.c., g.l.c., or combinations of these methods; have been used in the separation of some molecular species. However, none of these methods is presently capable of individual molecular species analysis of complex polar lipids both qualitatively and quantitatively.

Recent work has clearly indicated the need to study each individual molecular species in relation to respiratory

distress syndrome (RDS). For example, analysis of the fatty acid composition of the surface-active lecithins, both from premature infants and from healthy full-term infants, showed that in the prematures the principal surface-active lecithin was 1-palmitoyl-2-myristoyl PC whereas in the full-term infants it was dipalmitoyl PC[10,30]. After breathing commenced, the proportion of dipalmitoyl PC in the premature infants increased. Further investigation on different molecular species should help to clarify recent claims for other surface active species such as sphingomyelin (S)[52,53], phosphatidyl glycerol (PG)[54-56], phosphatidyl N,N-dimethylethanolamine (PDME)[53,57], phosphatidyl inositol (PI)[57,58] and lysophosphatidyl choline (LPC)[57].

Mass spectrometry seems to be an ideal tool for the purpose of analyzing intact molecular species of phospholipids crucial to maturation of fetal lung. Although conventional electron impact mass spectrometry (e.i.m.s.) has shown its tremendous potential for analyses of complex mixtures from biological sources, it has been estimated[59] that ca. 75% of the biological samples cannot be handled by this technique because of the lack of volatility and/or thermal stability. Earlier work has shown that intact phospholipids belong to this latter category.

Various techniques of partial hydrolysis and derivatization or analysis of pyrolysis or fragmentation

products have partially overcome this handicap.

The difficulty in vapor phase analysis of phospholipids is illustrated by Perkins et al[60]. Under suitable g.l.c. conditions, phosphoglycerides gave peaks with retention times identical to those obtained from the corresponding 1,2-diglycerides (as the result of cleavage of the phosphate bond at glycerol) and their acyl migration products, the 1,3-diglycerides.

Silylation of phosphatidyl cholines, phosphatidyl ethanolamines, phosphatidyl serines, phosphatidyl inositols and cardiolipin after chemical[61,62] or enzymatic hydrolysis[63] led to the formation of more volatile 1,2- and 1,3- diglyceride trimethylsilyl ether derivatives suitable for g.c.m.s. analysis. Although good structural information was obtained from the lipophilic part, detailed structure of the polar part was still lacking. Another approach to identify the "backbone" components of glycerophospholipid was achieved by deacylation of the fatty acids from the parent phospholipids. The resultant glycerophosphate esters were then isolated and analyzed as their trimethylsilyl derivatives by g.c.m.s.[64,65]. Karlsson and co-workers[66] determined the polar part of choline-containing phospholipids by selective quantitative demethylation of either the choline group in the intact molecule or the phosphoryl choline liberated by phospholipase hydrolysis. The resulting phosphoryl-N,N-dimethylethanolamine was

analyzed as a trimethylsilyl derivative by g.c.m.s. More recently, various techniques used in separation and characterization of polar lipids by means of g.c.m.s. have been extensively reviewed[67]. Different g.l.c. conditions and derivatives used to identify the compounds as well as e.i.m.s. fragmentation patterns were fully discussed.

The closest approach to date to a mass spectral analysis of intact phospholipids have been developed by Klein[68-70]. Molecular ions of underivatized phosphatidyl cholines were absent even when the electron energy was reduced to 14 eV but evidence for these molecular ions was obtained from metastable ions when the instrument was operated in defocussed mode. The low intensity of these ions and the considerable experimental difficulties inherent in operation of the instrument in the defocussed mode make this technique rather unattractive for extensive use. Similar results for other phospholipids were found by Boettger and Kelly[71].

In summary, if mass spectrometry is to provide detailed structural information about both the lipophilic part and the polar part of the molecules simultaneously, other milder ionization methods giving enhanced molecular ion intensity seem to be needed. Although isobutane chemical ionization mass spectrometry allows the detection of an ion equivalent to M+H for dioleoyl PC[72] with intensity as ca.

30% of the base peak, this result may reflect the tendency of that compound to rearrange into the more volatile methyl ester of the corresponding phosphatidyl N,N-dimethylethanolamine[73].

Since field desorption mass spectrometry (f.d.m.s.) was introduced by Beckey and his colleagues in 1969[74], it has opened a new era for the mass spectrometric analysis of underivatized, highly polar, nonvolatile and thermally labile compounds, such as phospholipids[75,76,77]; organic salts[78-81]; and many other biologically significant compounds[82-85].

In preparation for the application of f.d.m.s. to structural studies of the lecithin fraction of amniotic fluid including quantitation of dipalmitoyl PC, a survey of phospholipid behavior was undertaken, the results of which form the basis of this dissertation. The methodology necessary for the biomedical studies is under development in our laboratory. It involves the addition of a known amount of d_4-dipalmitoyl PC to the amniotic fluid sample as an internal standard, separation of lecithin fraction by preparative t.l.c., and comparison of the relative peak heights of the unknown and the standard recorded by repetitive scanning over a narrow mass range in the molecular weight region.

In addition to quantitation of the species most often linked to surfactant properties of amniotic fluid, the specificity and sensitivity of this technique offer promise

in the study of concentration changes of each individual molecular species of the various phospholipids in amniotic fluid at different gestation ages. This may provide a clear picture about the correlation between all surface-active phospholipids and fetal lung maturity in both biochemical and clinical aspects.

Other medical conditions related to lipid metabolism (atherosclerosis for example), which are difficult to analyze by conventional means, could present an interesting challenge for this powerful technique.

Before the actual analysis of various kinds of naturally occuring phospholipids in biological fluids is carried out, it is necessary to establish the basic fragmentation patterns for each species of known structure and develop isolation, purification, as well as mass spectral techniques for the quantitation of individual phospholipids, alone or in mixtures. In the present study, f.d. mass spectra of various synthetic phospholipids were determined, including 18 phosphatidylcholines (PC), 5 lysophosphatidyl cholines (LPC), 4 phosphatidyl ethanolamines (PE), 2 lysophosphatidylethanolamines (LPE), 1 phosphatidyl N,N-dimethylethanolamine (PDME), 2 phosphatidic acids (PA), 3 lysophosphatidic acids (LPA), 3 glycerophosphoryl amino alcohols, 3 phosphoryl amino alcohols, 5 sphingolipids, and other related compounds.

CHAPTER II

THE FIELD DESORPTION TECHNIQUE

A. Basic Principles

The field desorption concept has been known for a long time.[86] In principle, this method is similar to field ionization which has been reviewed comprehensively in several articles[83,87-91] and a monograph[92]. Physicochemical principles, analytical applications and problems of the FD method were also discussed by Beckey and Schulten.[82]

When organic molecules are deposited on an "activated" field anode (also called emitter or field emitter) from a solution or suspension, they will experience a force when a strong electric field (usually in the order of 10^7 - 10^8 V/cm) is applied, just as there is a force on the plates of a charged capacitor. This force can become large enough to remove an electron from the neutral molecule by the well-known quantum mechanic tunneling effect. The resulting positive ions are subsequently analyzed mass spectrometrically. This is the basis of f.d.m.s.

In common with f.i.m.s., this ionization does not involve major transfer of internal energy to the molecules. However, conventional f.i. has the disadvantage that sample vaporization is necessary prior to ionization. For those

thermally unstable substances, the energy required to overcome the crystal lattice forces is sufficient to cause cleavage of the weaker covalent bonds. In the f.d. method, on the other hand, ionization of the absorbed molecules and desorption of these ions from the field anode demand only a sufficient surface mobility of the molecules such that the substance to be ionized can diffuse to the sites where ionization takes place.

B. Field Anodes for f.d.m.s.

Fine wires or sharp metal edges are usually not capable of generating high enough electric field for f.i. or f.d. of most organic substances. Based on the fact that the field strength generated at a given voltage depends upon the shape of electrode (the smaller the radius of curvature, the higher the field produced), the field can be greatly enhanced by growing microneedles on the field anode.[93] This process of needle growth is termed "activation" or "conditioning" of an anode. Systematic studies have indicated that benzonitrile is the best activating reagent[92,94,95] and the degree of activation depends on the nature of activating reagent, the voltage, temperature, pressure and the radius of the anode.[95] The exact chemical nature of the microneedles has not been established but it is generally believed that they are semiconductors composed of organic polymers with

high carbon content.[94,96-98] Several activation methods[98,101] for the preparation of microneedles at low and high temperature have been developed. Beckey and Schulten[95,98,99] demonstrated that field anodes activated at higher temperature are resistant both chemically and thermally and are more suitable for f.d.m.s. It should be emphasized that f.d.m.s. of organic substances stands or falls on the quality of the ion emitter and strongly depends on the anode temperature.

As far as f.d.m.s. is concerned, it is recommended that microneedles should be as long as possible since a large surface area is then available for sample deposition. On the other hand, increasing microneedle length is accompanied by a decrease in the field strength at the emission sites and a corresponding decrease in the intensity of the ion currents produced. Therefore, needles of 20 - 40 μm in length have been proved to be the best compromise.[102]

Owing to the chemical and morphological structure of the microneedles grown on the surface of a field anode, some technical problems can arise:

1) In view of the fact that microneedles are organic semiconductors, at a given voltage the tip of a needle is at a lower potential than its base. The potential drop is bigger for thinner and longer needles. Thus, the ions formed at the tips will have slightly higher energy than those formed near the wire. Such an energy spread leads

to peak broadening in a single-focusing instrument. Consequently, an instrument with higher resolving power such as a double-focusing mass spectrometer is required for unit resolution above 500 - 600.[92]

2) It is generally believed that the thermal conductivity of the graphitic material of microneedles is smaller than that of metals, and as a result, the temperature is not identical at different points of the wire for a given current passing through the wire.[103] Firstly, the temperature drops from the centre towards the ends of the wire. This decrease in temperature corresponds to an additional heating current between 0.5 - 5 mA. Secondly, there is a temperature gradient along the microneedles, that is, the temperature of the microneedles near the carbonized tungsten wire surface is higher than at their tips. Winkler and Beckey[104] have recognized this problem and have explored the possibility of indirect heating of the wire by i.r. radiation. This improves the situation, but the question of local temperature gradient remains one of the poorly understood aspects of f.d.m.s.

C. Recent Advances in Solving f.d.m.s. Problems

In spite of the rapid development of f.d.m.s. as an established and useful analytical tool, several experimental difficulties have been encountered by the users of this

technique.[105,106]

1) Reproducibility:

The major problem of generating reproducible mass spectra from successive analyses of the sample has been a serious drawback of this technique. Generally speaking, f.d. mass spectra of a substance are less reproducible than mass spectra produced by other common ionization methods (e.g. e.i., c.i. or f.i.). It appears that the major cause is related to temperature of the anode. The best anode temperature (BAT, also called optimal field anode temperature) of a specific compound is defined as the temperature (or heating current) at which the molecular ion (or $[M + H]^+$) intensity is maximal and fragmentation is minimal. In the case of direct electrical heating, it is generally characterized by quoting the heating current passing through the emitter wire. Any one of the following parameters affecting the BAT may be the leading cause of the poor reproducibility of f.d.m.s.

a) Effective electric field:

The stronger the electric field, the lower the BAT required for f.d.

b) Solvent and pH:

Solvation of the molecules is another factor to be considered. The change of solvent, from organic to aqueous for example, and the change of pH may affect

the BAT, especially for those highly polar compounds and organic salts.

c) Distribution and thickness of the adsorbed layer:
By means of the commonly used dipping technique,[74] it is almost impossible to obtain an uniform distribution and thickness of sample layer on the field anode. Significant variations in BAT as well as f.d. mass spectra are then expected for differeht sample loads.

d) Chemical properties and morphological structures of microneedles:
Although prepared under the same activation conditions, microneedles on each emitter may have different lengths and structures. For a series of measurements of a given compound with different emitter wires, variation in BAT is even greater than that with repeated sampling on the same emitter.[107] Such a variation is responsible for poor reproducibility of f.d. mass spectra with different emitters.

In addition, experience has revealed that precise and reproducible control of emitter heating is the most important variable in production of quality f.d. mass spectra and reproducible results for successive analyses of the same sample. The standard method of emitter heating by manual control of a ten-turn helipot (as used in the Varian CH5 instrument) cannot conveniently serve this purpose.

Recently, this shortcoming has been overcome with a solid state emitter current programmer (ECP).[107] This device can precisely regulate the heating current to within 0.1 mA at any level between 0 - 80 mA and also provides a linear temperature programming from chosen initial to final current at a preset rate.

Under normal f.d. operating conditions, the total ion current produced is usually smaller than that using other ionization methods, especially for those compounds with very low ionization efficiency. Statistical fluctuation of such a low ion current is another handicap for producing reproducible spectra. Rapid anode heating may enhance the ion current intensity, but only at the expense of shortened time of measurement because of the limited quantity of substance available. These times are between several seconds and several minutes. However, this dilemma can be overcome in part by integrated ion detection with photographic plates or with electrical recording using computer controlled multiscanning and integration techniques. Increasing the total transmission (which is defined as the ratio of the current measured at the first dynode of the secondary electron multiplier (SEM) ion detector to the total ion current emitted from the field anode) is another possible approach towards this problem. This can be achieved by coupling a f.d. ion source with a quadrupole

mass analyzer.[108] Though f.d. quadrupole mass spectrometry is still in its infancy and involves many technical problems, it could offer a new way to expand the detection limit of f.d.m.s. and become important in special areas of f.d. analyses.

2) Quantitative determinations:

F.d.m.s. has been shown to be suitable for qualitative analysis of mixtures, but not much work has been done on quantitative determination of such mixtures. In this laboratory trimethylammoniumbenzenesulfonates with varying deuterium content have been analyzed[109] as well as mixtures containing varying ratios of the cancer drug cyclophosphamide and its d_4-analogue.[110] These studies indicate that quantitative f.d.m.s. with electrical recording is feasible, but that ion statistics impose some limitations on precision and on dynamic range. Two recent publications, dealing with quantitative sample loading and continuous heating control, markedly improve the potential of f.d.m.s. for quantitative analysis.

Sample loading onto the emitters has been carried out by dipping technique[74] in routine analysis. The activated emitter wire is dipped into a solution of the sample, and when it is subsequently removed a droplet of solution may adhere to the microneedles. After the solvent is evaporated, the procedure is repeated several times until

sufficient amount of sample is deposited. This technique has proved to be very convenient, however, it presents two limitations. The first is the sample size. A relatively large volume of solution is required and only a small fraction of the total sample is deposited on the wire. Although the solution remaining can be used for further dipping or recovered after evaporating the solvent, the technique is inapplicable when only a small quantity (less than ca. 1 mg) of material is available. The second limitation is nonquantitative sample deposition. Experiments revealed that for a given sample of a given concentration, the amount of material deposited on the wire by the dipping technique may vary by a factor of ten.[111] These limitations have been overcome recently by the "freezing technique". A given volume of solution can be quantitatively transfered from a microsyringe to the cooled emitter by freezing. Using this technique, sample consumption can be reduced and the sample deposition becomes more reproducible. Both of these factors can expand the application of f.d.m.s. to include analyses of trace quantities (sub-microgram) and quantitative measurements.

A second problem associated with quantitative determination by f.d.m.s. is the effect of anode temperature. As has been mentioned in the previous section, there is a

BAT for each compound. At a constant anode heating current, the f.d. signals are not ordinarily proportional to the mole fraction of each component in a mixture (a case analogous to fractional distillation). Moreover, the quantity of each component in a mixture may not be proportional to the total ion intensities of all the ions generated from that component. The intensity of each ion depends upon many parameters in addition to what might be considered an inherent ionization efficiency (such as effective field strength, nature and structure of micro-needles on the wire, thickness of the sample layer and the anode heating rate). Until now, quantitative determination of a specific constituent in a complex mixture has relied upon a closely related compound (such as a deuterium - labelled analogue) as an internal standard. Comparison of ion intensities between unknown and standard for quantitative measurement then assumes that both of them behave the same. The introduction of emitter current programming (ECP) promises to place quantitative measurements on much firmer ground. Repetitive scanning of the m.s. under computer control during linear ECP allows the production of reconstructed mass chromatograms of ions (e.g. $[M]^{+\cdot}$ or $[M+H]^{+}$) characteristic of the unknown and the internal standard.[107] Assuming that the areas under the peaks of these ions are proportional to the concentration of the

compounds deposited on the emitter wire, the concentration of a specific component in a mixture can then be determined by comparing the ratios of its peak areas to those of the internal standard.

3) Alkali metal ion contaminations:

Interference of metal ions, particularly the widely distributed sodium and potassium ions, in the f.d. process has been a serious difficulty in running naturally occurring samples. Presence of these ions may result in a characteristic pattern of low detection sensitivity, high emitter current, unsteady ion beam current and poor reproducibility. In certain cases these impurities can lead to a complete failure to obtain a mass spectrum of organic compound. The exact level of metal ions that will cause deleterious effect on an f.d.m.s. sample is quite difficult to establish. It appears to depend mainly on the chemical and physical properties of the compound itself. Some compounds can tolerate a fairly large amount of metal ion impurities with no apparent effect, others may form complexes with the metal ions to give an intense peak (M + metal ion) in the f.d. mass spectrum. In contrast, some are highly sensitive to metal ions even at the ppm level.

There are many aspects of this problem not clearly understood, not the least of which is the mechanism by

which the metal ions cause trouble. Several of the points made by Schulten and Rollgen[81] concerning f.d.m.s. of inorganic salts may be applied to the present problem.

a) A preferentially adsorbed salt layer between the anode and the organic sample may reduce the effective electric field on the emitter surface by its high dielectric constant and thus diminish the ion-formation probability. In order to induce emission, the ionization sites at the tip of the microneedles must be freed by heating the emitter to sinter the salt layer. Thermal degradation of the organic sample may result.

b) Ionization of inorganic salts, NaCl for instance, leads to the production of a radical which in turn may be bound to the emitter surface and "deactivate" the emitter.

$$NaCl \xrightarrow{-e} * Cl + [Na]^+$$

(* is a radical site on the surface of the emitter, e.g. a C-Cl bond)

c) Another possible cause of this effect is strong binding of the organic substance in the lattice of the inorganic salt.

Although any of these mechanisms could give a satisfactory explanation for the deleterious effect of metal ions, their relative importance still has not been established.

Several methods have been employed to remove the interfering metal ions from f.d.m.s. samples. Although gel chromatography[102] or dialysis is capable of serving this purpose, such procedures are rather time consuming and the latter is wasteful of sample. Addition of a macrocyclic ligand, such as crown ether or cryptand,[113] to the samples contaminated with alkali metal ions prior to f.d.m.s. analysis, has been shown to result in a reproducible, smooth f.d. process at moderate anode temperature. Recently, in connection with our nucleoside work (Part II of this dissertation), it was found that solvent extraction of the aqueous sample solution by a solution of crown ether dissolved in an organic solvent is also capable of reducing the alkali metal ion concentrations, at least to a tolerable level.

CHAPTER III

EXPERIMENTAL

A. Instrumental

Mass spectra were obtained on a Varian CH-5 DF mass spectrometer equipped with a combined f.d./f.i./e.i, source. Some of the spectra were recorded through an INCOS Model 2000 computer interfaced with the m.s. Other data were recorded on photographic paper calibrated against the e.i. spectrum of perfluorokerosene obtained under identical scanning conditions. The anodes are 10μ tungsten wires spot-welded on supporting posts and activated in a Varian apparatus in a manner similar to that described by Beckey.[98] Samples dissolved in chloroform (ca. 5 mg/ml) were deposited on the activated anode by the dipping technique.[74] After excess solvent had evaporated, the anode carrying the sample was introduced into the cool ion source (generally 70°-100°C) through a vacuum lock. When vacuum better than 10^{-6} torr was restored anode voltage of +3 kV and cathode voltage of -7 to -8 kV were applied. Admission of acetone through the batch inlet system was used to optimize the f.i. beam current by adjusting the focusing elements. Anode heating was increased until a steady ion beam was obtained on the total ion beam monitor, source elements were fine tuned on sample ion if necessary, and the magnet

scan was then commenced. Nominal resolution was 1000 and 1500 (10% valley definition) for oscillographic and computer recording respectively. The secondary electron multiplier was set at 2 kV for manual operation and at 1.75 kV for computer acquisition. The magnet was scanned linearly from m/e 10-950 at an average speed of 25 a.m.u./s. for manual operation and from m/e 900-10 every 20 s. under computer control. After each sample, the anode current was gradually increased to its maximum value (50 mA) to clean the wire before the next sample was loaded.

B. Chemicals

Dibutyroyl PC, dioctanoyl PC, dipalmitoyl PC, distearoyl PC, dilinoleoyl PC, dilinolenoyl PC, diarachidonyl PC, palmitoyl LPC, stearoyl LPS, oleoyl LPC, linolenoyl LPC, arachidonyl LPC, dilauroyl PE, dipalmitoyl PE, dioleoyl PE, palmitoyl LPE, oleoyl LPE, dipalmitoyl PDME, dicaproyl PA, dipalmitoyl PA, caproyl LPA, palmitoyl LPA, oleoyl LPA, dipalmitoyl phosphatidyl glycerol, dipalmitoyl PS, N-palmitoyl dihydrosphingosine were purchased from Serdary Research Laboratory, London, Ontario.

Dicaproyl PC, 1-palmitoyl-2-myristoyl PC, 1-palmitoyl-2-oleoyl PC, 1-oleoyl-2-palmitoyl PC, 1-palmitoyl-2-linoleoyl PC, dioleoyl PC, distearoyl PE, glycerophosphoryl choline, glycerophosphoryl ethanolamine, glycerophosphoryl serine, sphingosine, psychosine, kerasin, were purchased from

Supelco, Inc., Pennsylvania.

Dilauroyl PC, dimyristoyl PC, dihexadecyl ether phosphatidyl choline, 1-(octadec-9-enyl)-2-hexadecyl ether PC, were obtained from Calibiochem. Inc., San Diego, Calif.

Phosphoryl choline chloride (calcium salt), and phosphoryl ethanolamine were obtained from Sigma Chem. Co., St. Louis.

Dipalmitoyl d_4- and d_9-PC were synthesized[77] and kindly supplied by Dr. G.N. Subba Rao of this department.

All solvents and other reagents were ACS grade and were used without further purification. Although all synthetic phospholipids were claimed to have a purity better than 99% by the manufacturers, a purity check was carried out by TLC analysis. If more than one spot was detected, attempts were made to purify them by chromatography on silicic acid, or acid-treated Florisil or Bio-gel HTP.

C. Chromatographic Procedures

1) Analysis by t.l.c.[114-116]

Silica gel H (without $CaSO_4$ binder; Supelco) plates (ca. 0.25 mm thickness) or REDI-COAT (Supelco, Inc.) plates, activated at 110°C for 2 hours before use, were used for purity check of purchased synthetic phospholipids and for the examination of eluates from columns. Phospholipids and the necessary standards dissolved in

either chloroform or chloroform-methanol were applied to the plate. Chloroform-methanol-water (65:25:4) was used as developing solvent. After chromatography, individual lipids were visualized by either iodine vapour, or phosphate stain[42], or 0.2% 2',7'-dichlorofluorescein (Eastman, Kodak Co.) in 95% ethanol under UV light.

2) Silicic acid chromatography[42,43] of PC

Silicic acid (100 mesh, A.R. grade, Mallinckrodt Chem. Co.), 1.2 g., was activated at 110°C overnight, slurried in $CHCl_3$, and poured into a disposable column (0.7 cm diameter).

A column of 0.7 x 15 cm. thus formed was washed with about 100 ml of $CHCl_3$. The phospholipid (ca. 5 mg dissolved in minimum amount of chloroform) was applied to the column which was subsequently eluted with $CHCl_3$:MeOH (7:3 v/v). Fractions (2 ml) of the eluate were collected and concentrated under a gentle stream of N_2. Column fractions were identified by comparison with standard phosphospholipids on silica gel H microslide plates following the procedures described in section (1).

3) Hydroxylapatite (Bio-gel HTP) chromatography[117]

a) Column preparation

Twenty grams of Bio-gel HTP (Bio-rad Lab., Richmond, Calif.) was washed with 3 x 150 ml methanol, 2 x 150 ml acetone and 2 x 100 ml diethyl ether.

After each washing, the supernatant was decanted. Residual ether was removed in a stream of N_2. The ether-free adsorbent was activated at 110°C overnight and cooled in a desiccator. The adsorbent (0.5g.) slurried in chloroform was transferred to a column (0.5 x 10 cm). The column bed was then washed with 100 ml of $CHCl_3$.

b) Chromatography of phospholipids

Phospholipid (5-10 mg) dissolved in ca. 0.5 ml chloroform or chloroform-methanol mixture (2:1 v/v) was applied to the column. The lipids were eluted from the column by the following solvents: (i) 5 ml acetone-methanol (9:1); (ii) 15 ml acetone-methanol (7:3)——lecithin fraction; (iii) 15 ml acetone-methanol (5:5)——sphingomyelin fraction; (iv) 20 ml methanol——PE. One ml fractions of the eluate were examined by t.l.c. on silica gel plates.

4) Acid-treated Florisil Chromatography[42] of Acidic phospholipids

Approximately 5 g. of acid-treated Florisil (Serdary Lab., London, Ont.) activated at 120°C overnight was slurried in chloroform and poured onto a disposable column (0.7 x 25 cm). After the column bed was washed with 70 ml of $CHCl_3$, ca. 10 mg of phosphatidic acid or lysophosphatidic acid dissolved in $CHCl_3$ was applied to

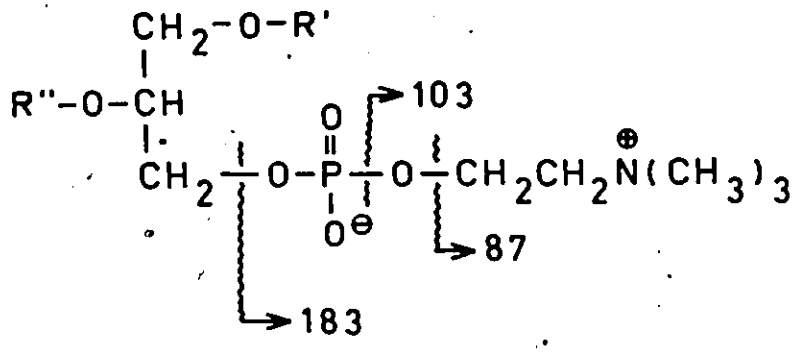
the column, and then eluted at a flow rate of ca. 1 ml/min. with (1) 50 ml $CHCl_3$ (2) 50 ml CH_3Cl methanol = 9:1 (3) 50 ml $CHCl_3$:MeOH = 1:1.

CHAPTER IV

RESULTS AND DISCUSSION

A. Phosphatidyl Cholines† (PC)

In preparation for the application of f.d.m.s. to analytical problems, 15 synthetic diacyl PCs (I-XV) and 2 dialkyl ether PCs (XVI,XVII) with known fatty acid composition have been characterized (Chart 1).



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†The proper name is 1,2-diacyl-sn-glycero-3-phosphoryl choline as recommended by IUPAC-IUB Commission. Other names such as D/L-α-phosphatidyl choline, D/L-3-phosphatidyl choline, R/S-3-phosphatidyl choline and lecithin are widely adopted by various workers. Since the stereochemical numbering (sn) system is too cumbersome to use with regularity in this dissertation, and since no conclusions are based on stereochemistry, we shall use the term "phosphatidyl choline" (PC).

Chart 1.

Compound	Fatty Acids	Name	Mol. Wt.
I	R'=R"=C_3H_7CO	Dibutroyl PC	397.3
II	R'=R"=$C_7H_{15}CO$	Dioctanoyl PC	509.4
III	R'=R"=$C_9H_{19}CO$	Dicaproyl PC	565.5
IV	R'=R"=$C_{11}H_{23}CO$	Dilauroyl PC	621.5
V	R'=R"=$C_{13}H_{27}CO$	Dimyristoyl PC	677.5
VI-d_o	R'=R"=$C_{15}H_{31}CO$	d_o-Dipalmitoyl PC	733.6
VI-d_4	R'=R"=$C_{15}H_{31}CO$	d_4-Dipalmitoyl PC	737.6
VI-d_9	R'=R"=$C_{15}H_{31}CO$	d_9-Dipalmitoyl PC	742.6
VII	R'=$C_{15}H_{31}CO$ R"=$C_{13}H_{27}CO$	1-Palmitoyl-2-myristoyl PC	705.6
VIII	R'=$C_{15}H_{31}CO$ R"=$C_{17}H_{33}CO$	1-Palmitoyl-2-oleoyl PC	759.6
IX	R'=$C_{17}H_{33}CO$ R"=$C_{15}H_{31}CO$	1-Oleoyl-2-palmitoyl PC	759.6
X	R'=$C_{15}H_{31}CO$ R"=$C_{17}H_{29}CO$	1-Palmitoyl-2-linoleoyl PC	757.6
XI	R'=R"=$C_{17}H_{35}CO$	Distearoyl PC	789.6
XII	R'=R"=$C_{17}H_{33}CO$	Dioleoyl PC	785.6
XIII	R'=R"=$C_{17}H_{31}CO$	Dilinoleoyl PC	781.6
XIV	R'=R"=$C_{17}H_{29}CO$	Dilinolenoyl PC	777.6
XV	R'=R"=$C_{19}H_{31}CO$	Diarachidonyl PC	829.6
XVI	R'=R"=$C_{16}H_{33}$	Dihexadecyl ether PC	705.5
XVII	R'=$C_{18}H_{35}$ R"=$C_{16}H_{33}$	1-(Octadec-9-enyl)-	731.6

Results obtained at their BAT are presented in Appendix I. The f.d. mass spectrum of dicaproyl PC is shown in Fig. 1 as an example. As a general feature for f.d.m.s., all PCs except II, VII, XI, and XII displayed the $[M+H]^+$ ion as the base peak.* Increasing anode temperature also induces the production of structurally relevant fragments which can be used for further characterization of the compound.

An examination of f.d. mass spectra of various PCs containing fatty acid residues with different chain length (C_4 to C_{22}) and degree of unsaturation (from fully saturated to tetraenoic) reveals that their behaviour is independent of the fatty acid residues present. In addition, their BATs are also not affected by the acyl chains. These spectra show that the polar phosphoryl choline moiety plays the most important role in fragment ion and ion cluster formation, while the hydrocarbon chain has little effect on it. The difference between these two groups is perhaps best appreciated by consideration of their solid-state properties in the condensed phase on the emitter. In spite of the very limited data on X-ray structure of intact phospholipids available in the literature, preliminary X-ray studies of 1,2-dilauroyl PE[118] suggest the following important structural features: (a) the two hydrocarbon chains of the fatty acid residue present in each phospholipid

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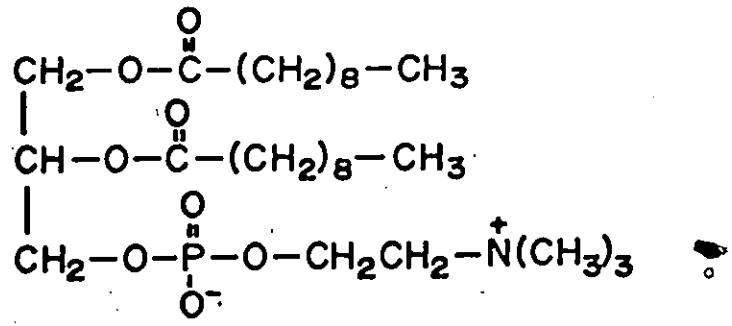
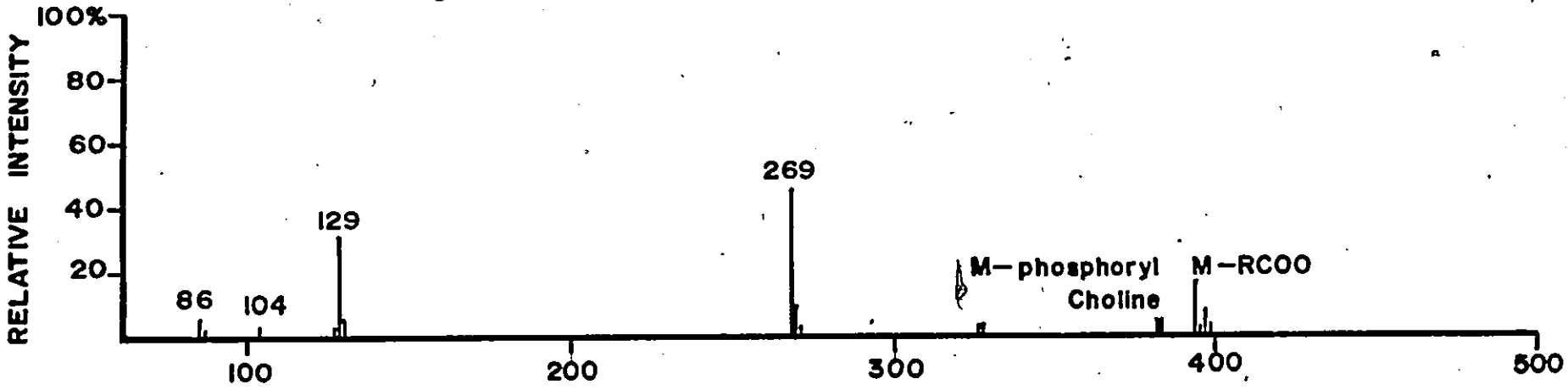
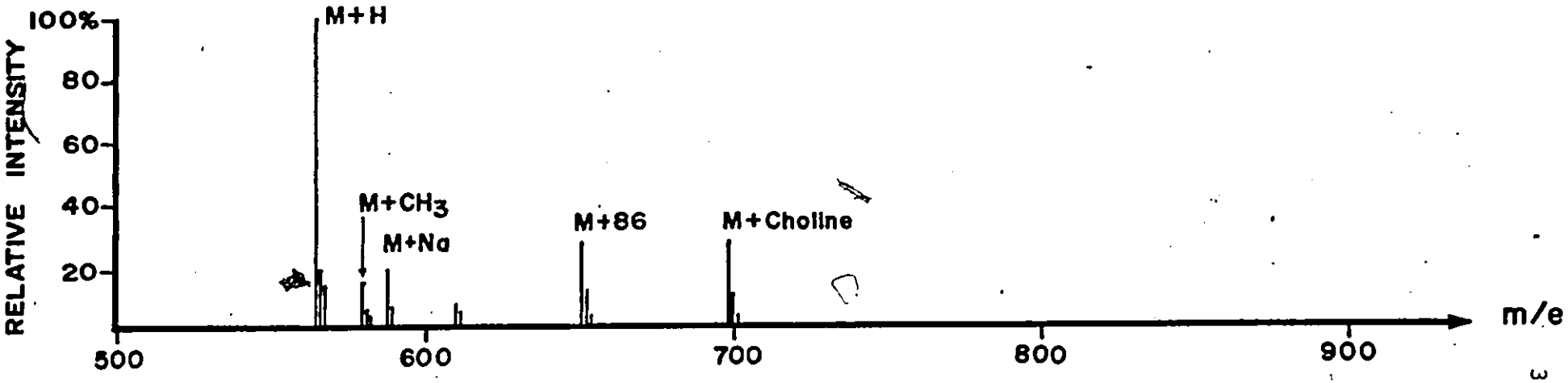
*Even for these compounds, the spectra are normalized to $[M+H]^+ = 100\%$ (see Appendix I).

Fig. 1. Field Desorption Mass Spectrum of Dicaproyl Phosphatidyl Choline.

FDMS OF DICAPROYL PHOSPHATIDYL CHOLINE (21mA) M.Wt. 565 SCAN#2







molecule are pointing in the same direction and are parallel to each other; and (b) the polar portion of the molecule containing the phosphate and the amino group appears to be in line with the hydrocarbon chains. The first point is not surprising since, beyond a certain chain length, the hydrophobic interaction favours the parallel hydrocarbon chain packing. Such orientation is important because according to these findings, the solid-state structure of phospholipids can be regarded as "infinite" sheets of phospholipid bilayers with the polar groups organized in sheets and with all the hydrocarbon chains of adjacent lipid molecules lying parallel as shown in Fig. 2. This is somewhat similar to the arrangement of lipid molecules which has been suggested to occur in many biological membrane systems. Such sheet-like arrays or lamellae formation (especially favourable for zwitterionic phospholipids such as PC) is believed to be one of the minimum energy configurations. Phospholipids aggregated in this manner decrease the intermolecular distance thus favoring interaction between adjacent polar groups. As a result, rearrangement and association reactions as well as intermolecular methyl transfer processes become feasible.

Since dipalmitoyl PC (VI) has been shown to be the major component in the amniotic fluid lecithin fraction, it was chosen as an example for extensive studies on structural elucidation of phospholipids by f.d.m.s. Field desorption

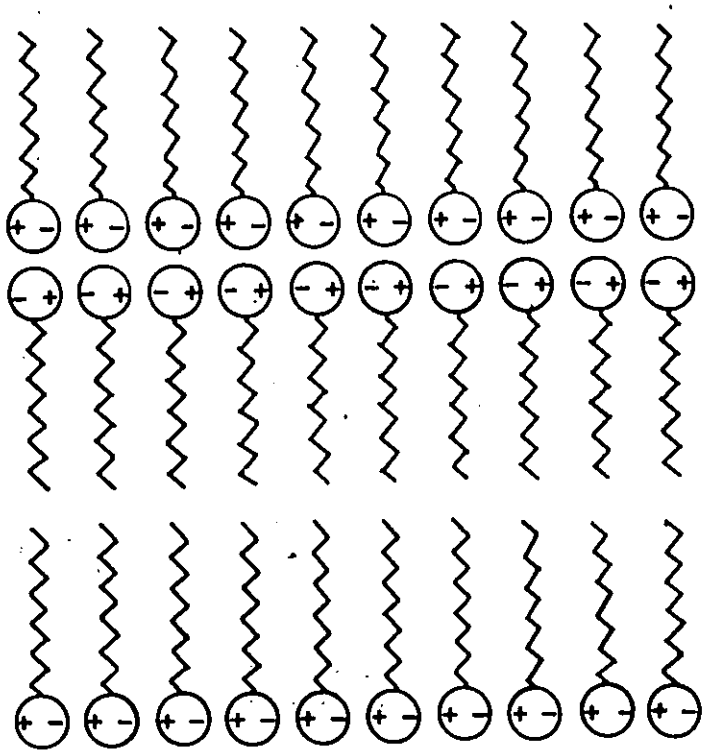


Fig. 2. Diagrammatic representation of a proposed phospholipid solid-state structure.

mass spectra of dipalmitoyl PC- d_0 (VI-d_0), -(methylene-d_4) (VI-d_4) and -(methyl-d_9)(VI-d_9) are presented in Table 1. Comparison was made of spectra of these three compounds in order to establish the composition of the major cleavage, rearrangement and association ions. The organization of Table 1 permits comparison in horizontal rows of similar ions even where they differ in isotopic composition.

Table 1

Comparative f.d.m.s. of Dipalmitoyl Phospatidyl Choline-d_0,-d_4, -d_9.[a,b]

d_0		d_4			d_9		
m/e	rel. int. 18 mA	m/e	rel. int. 20 mA[c]	rel. int. 21.5 mA	m/e	rel. int. 20 mA	rel. int. 22 mA
86	4.0	89		3.3	95		47
104	9.3	108	2.0	5.0	113		46
130		137		6.0	139		7.1[d]
239		239		3.3	230	7.3	
269	4.4	277		58	287	3.9	100
					305		26
550	30	550	3.4	53	550	25	2.7
551	23	551		43	551	44	3.1
552	7.1	552		12	552	13	1.8
553	2.2	553		1.3	553	3.7	1.3
734	100	738	100	100	743	100	18
735	46	739	46	46	744	41	10
736	7.5	740	12	9.0	745	7.3	2.7
		741	2.5	1.3	746	0.7	1.3
748	2.6	752	2.9	20	760	1.1	17
756		760	17	7	765	2.4	

...cont'd

Table 1 (cont'd)

[a]Ions up to m/e 800 with relative intensity > 3% in at least one set of scans are reported. Ions differing only in deuterium content are included in the same horizontal row. Tentative assignments above m/e 800 are mentioned in the text.

[b]Each vertical column of data is headed by the heating current at which it was obtained. These single scans do not generally permit quantitative comparison of ion intensities.

[c]The absence of m/e 89, 137, 239, 277, 551, 552 and 553 is likely related to the low total intensity of this spectrum and poor ion statistics rather than any more fundamental cause.

[d]A more intense ion (26% of the base peak) is present at m/e 138, presumably originated from a hydrogen loss (Scheme 2).

In general, ions produced from PC under f.d.m.s. conditions fall into three categories:

(1) The first category involves those ions resulting from the direct bond cleavage or rearrangement of the choline or phosphoryl choline moiety. Their proposed structures† are illustrated in Scheme 1. Thermally or field-induced rearrangement taking place in the condensed phase on the surface of the emitter is believed to be responsible for the formation of these ions.

The ion found at m/e 86 for all unlabelled compounds and corresponding ions at 89 and 95 in

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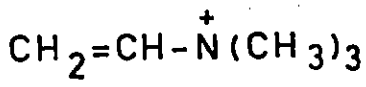
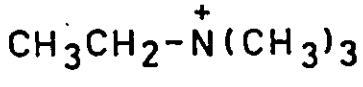
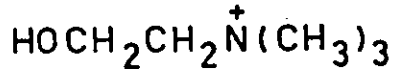
†These structural rationalizations and others which appear later in this dissertation are based upon comparison among the various molecules studied, particularly the labelled dipalmitoyl species. While this procedure allows reasonable assignments to be made, it is important that the limitations on the accuracy of these assignments be understood. Besides the evidence obtained from comparison of spectra, considerable judgement based on knowledge of mass spectral and solution chemistry was applied to development of these schemes. While this approach should be much less hazardous here than for e.i.m.s., there are no doubt alternative modes of ion formation which have not been considered or presented.



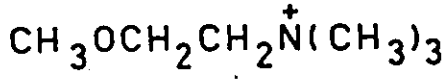
m/e 86



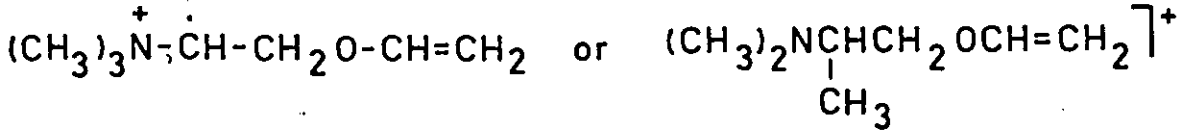
m/e 88



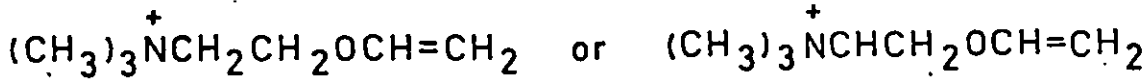
m/e 108



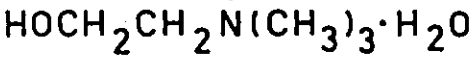
m/e 118



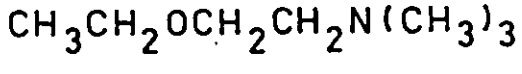
m/e 129



m/e 130

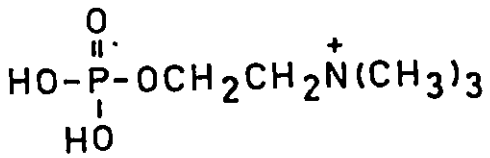


m/e 122

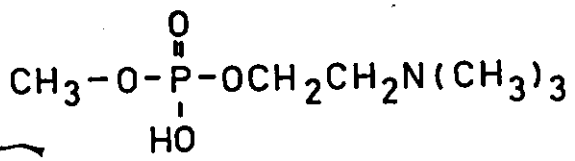


m/e 132

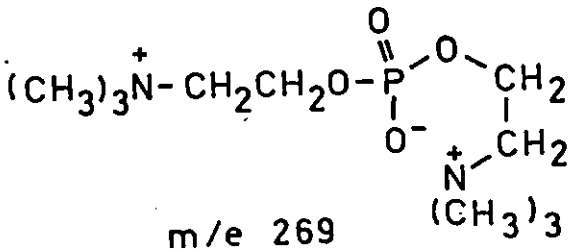
Scheme 1. Proposed Structures for Some Characteristic Fragment Ions in f.d. Mass Spectra of PCs.



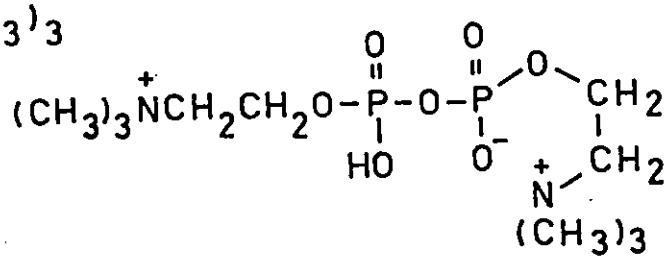
m/e 184



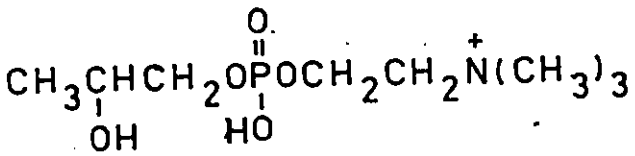
m/e 198



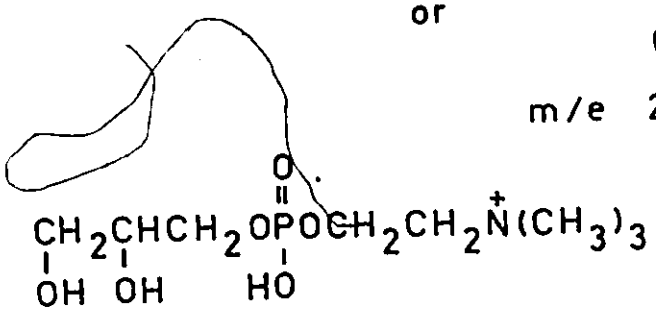
m/e 269



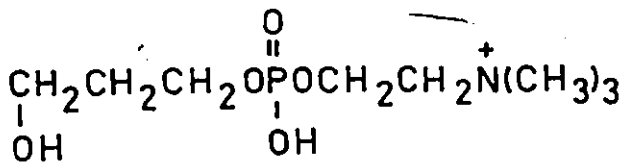
m/e 349



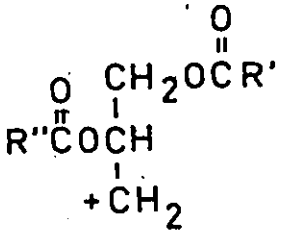
or



m/e 258



m/e 242



[M-phosphoryl choline]$^+$

Scheme 1 (cont'd)

VI-d_4 and VI-d_9 spectra respectively is most reasonably assigned to the dehydration product of choline. The fragmentation scheme is shown in Scheme 2. An ion at m/e 88 is equivalent in weight to structure i or ii. The former may be formed via bond rupture of the phosphate ester bond (as shown in Scheme 2) accompanied by hydrogen transfer while the latter may result from nucleophilic attack of the choline hydroxyl oxygen on the quaternary ammonium ion followed by demethylation. Although the m/e 92 ion in VI-d_4 is consistent with both structures, only structure i can account for the ion at m/e 97 observed in some scans of high temperature f.d. mass spectra of VI-d_9, which indicates the presence of three methyl groups rather than two as in structure ii. The assignments of the ion m/e 104 to the choline[†] cation (confirmed by the presence of the ions at m/e 108 and 113 in VI-d_4 and VI-d_9 mass spectra) and m/e 184 to phosphoryl choline[†] cation are

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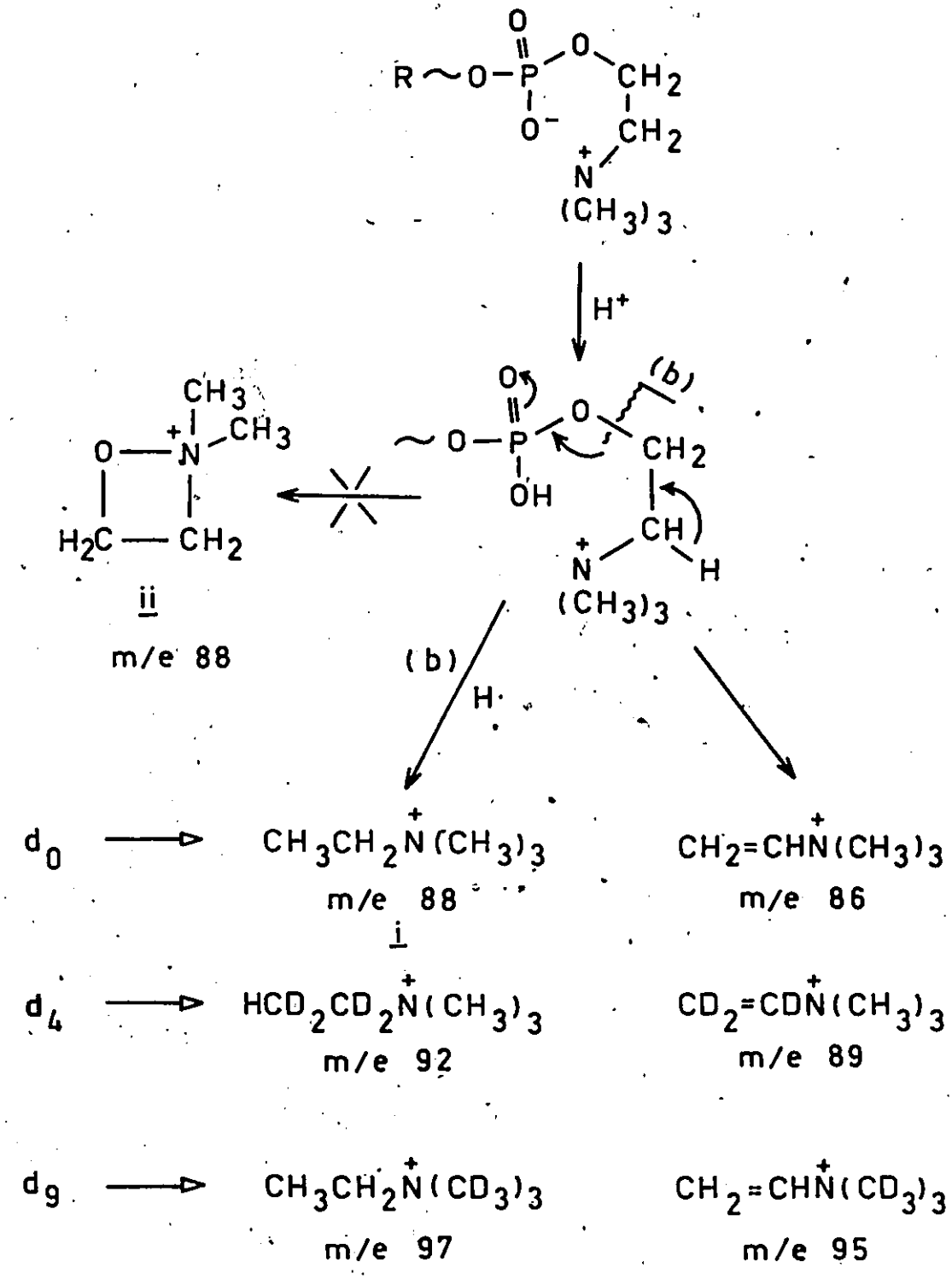
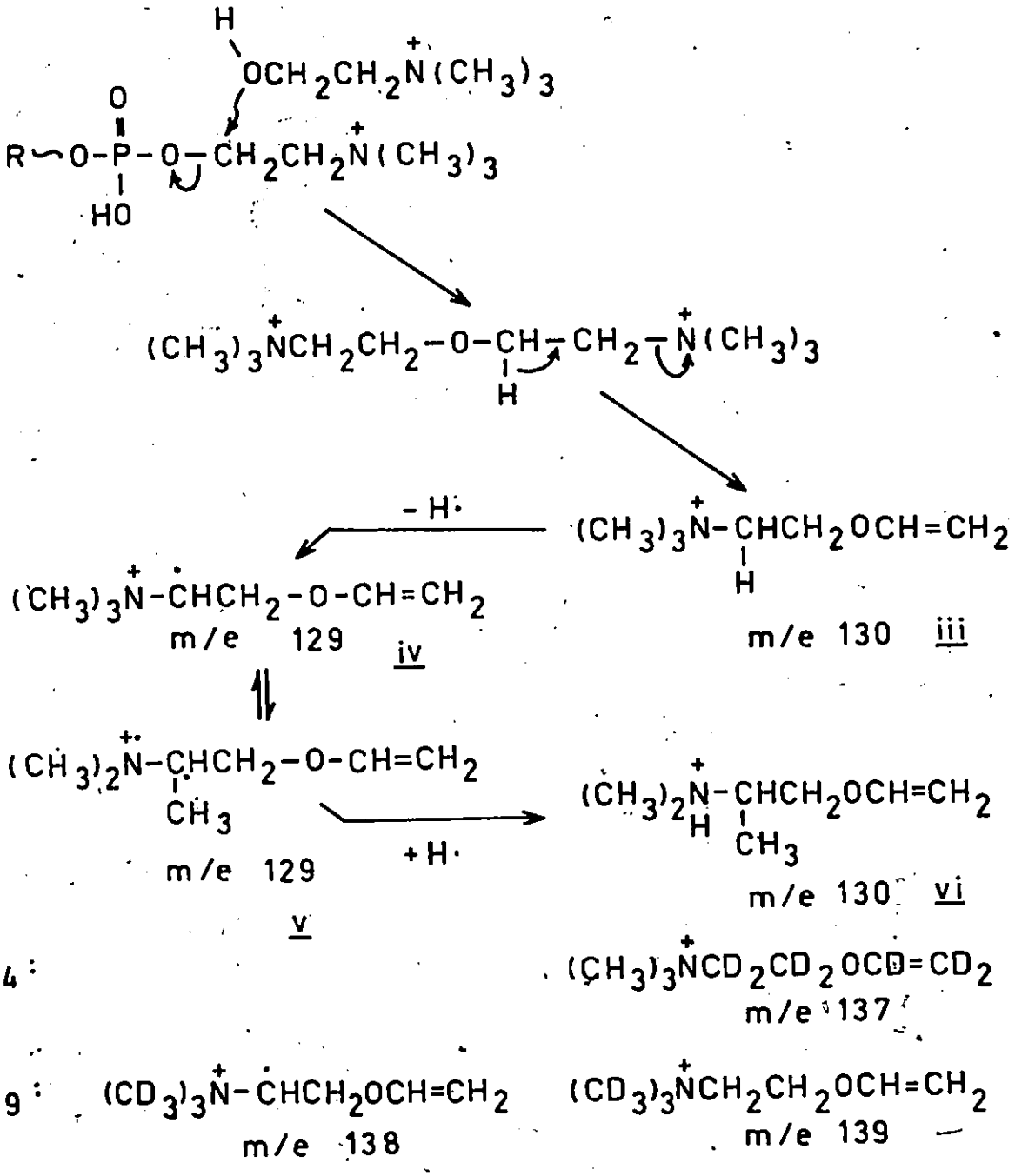
[†]Hereafter, the terms "choline" and "phosphoryl choline" will refer to the cationic species, otherwise the anion (eg. choline chloride, phosphoryl choline bromide) will be specified to represent the neutral molecule.



Scheme 2. Rationalization of the Formation of m/e 86, 88 Ions in f.d. Mass Spectra of PCs.

straightforward. The formation of these ions by simple bond rupture concomitant with proton transfer permits rapid identification of the polar part of the molecule. However, the contribution of impurities to the high relative abundance of these ions in some spectra cannot be excluded.

Significant ions at m/e 129 and 130, common in f.d.m.s. of a number of PCs containing a fatty acid chain longer than octanoic acid, have been assigned structures. The suggested mode of formation of these ions (Scheme 3) includes the formation of a dicholine ether as an intermediate which in turn expels one of its quaternary ammonium ions by Hofmann elimination and gives rise to the ion iii. Subsequent loss of a hydrogen atom from the carbon atom next to the quaternary ammonium ion leads to the formation of a radical iv or the formation of v after methyl migration. The latter ion (v) may undergo a further hydrogen transfer to bring the mass number back to 130 (ion vi). Considering the residence time of the focussed ions within the f.d. source (10^{-14} to 10^{-12} s. as compared to 10^{-14} to 10^{-6} s. under e.i. conditions),[96,119]



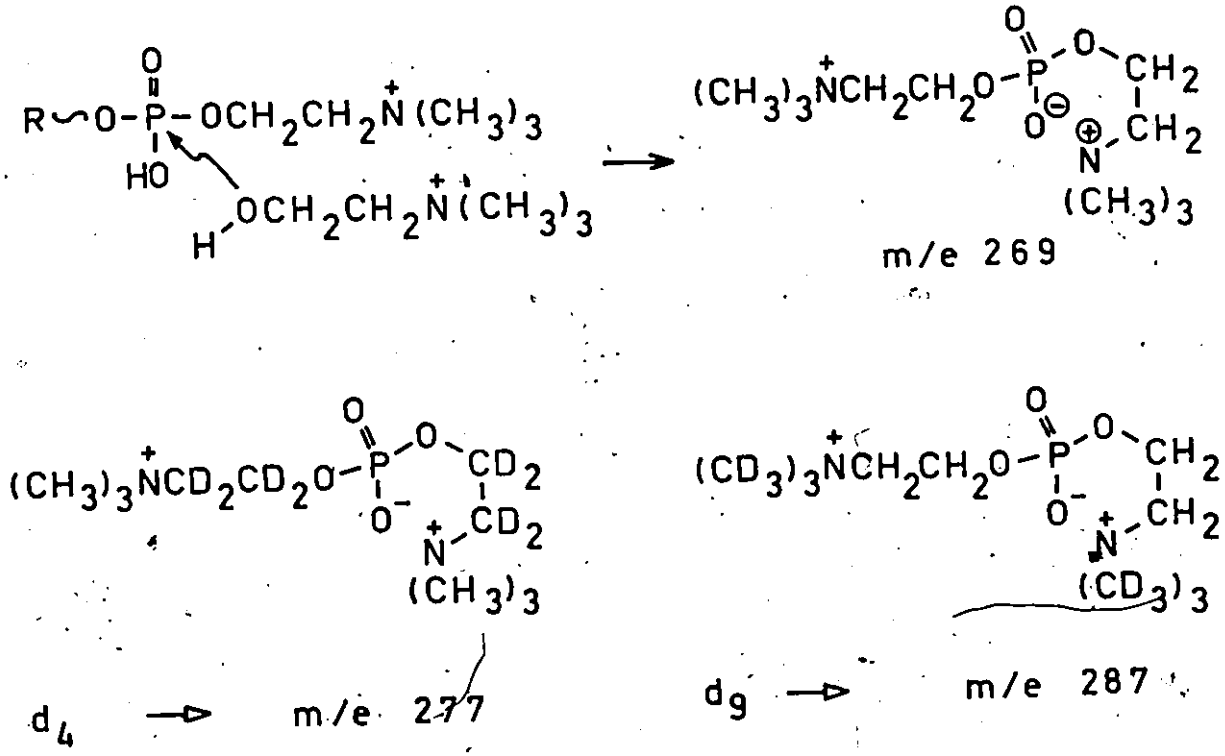
Scheme 3. Rationalization of the Formation of m/e 129 and 130 Ions in f.d. Mass Spectra of PCs.

such multistep rearrangement processes could not occur after f.d. Most of the rearrangement products seen therefore, must result from thermally and/or field-induced surface reactions between closely packed molecules adsorbed on the emitter surface prior to ionization. Surface reactions of this type still have not been fully understood although some simple molecules have been investigated with respect to the principal reaction mechanisms by means of f.i.m.s.[120] or pulsed f.d.[121]

The proposed structures (iii) and (vi) for the signal at m/e 130 are consistent with its shift to m/e 137 (VI-d_4) and 139 (VI-d_9). The high temperature VI-d_9 spectrum allows rationalization of the origin of the m/e 129 peak in f.d.m.s. of unlabelled compounds. However, the corresponding peak was not registered in the case of VI-d_4. An isotope effect may provide a reasonable explanation to account for the absence of the m/e 135 peak in the f.d.m.s. of VI-d_4. Calculation of the variation of isotope effect with internal energy predicts that high isotope effects should be observed at ion

energies just above the threshold for decomposition.[122] This condition is fulfilled in the case of field-induced decomposition. The production of the species iv and v which require a C-D bond cleavage may thus be hindered.

Ions at m/e 269 (all d_0-compounds) as well as 277 (VI-d_4) and m/e 287 (VI-d_9) are readily attributed to species containing two cholines. These ions are assigned the structure of dicholine phosphate which could be formed as shown in Scheme 4.



Scheme 4. Rationalization of the Formation of Dicholine Phosphate (m/e 269) in f.d. Mass Spectra of PCs.

The effect of anode temperature on the relative intensity of the m/e 269 ion in f.d.m.s. of dimyristoyl and dioleoyl PC is illustrated in Table 2.

Table 2

Effect of Emitter Heating Current (e.h.c.) on the Relative Intensity of m/e 269 Ion

a) Dimyristoyl PC

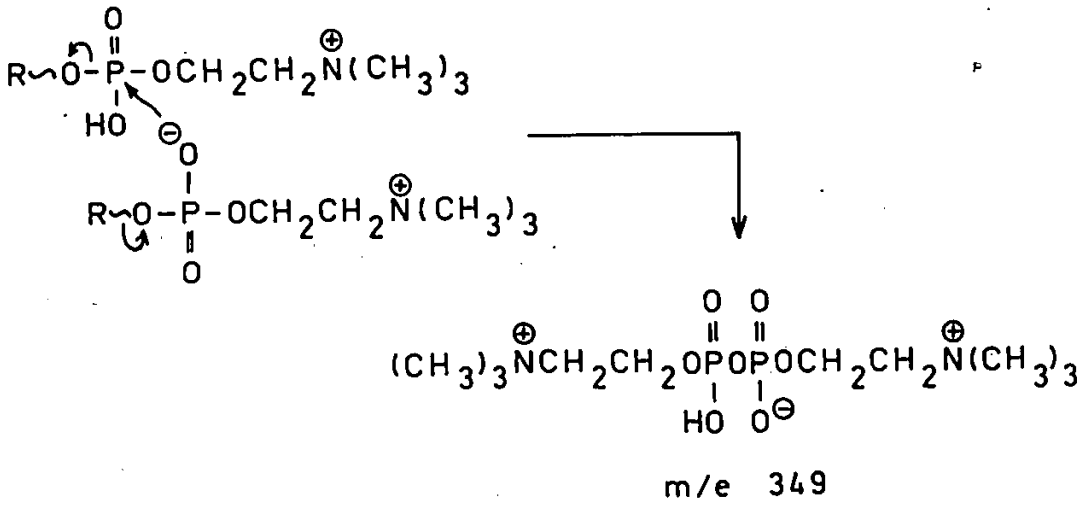
e.h.c. (mA)	18	18.5	19	19.5	20	20.5
rel. int.(%)	3	10	13	17	20	29

b) Dioleoyl PC

e.h.c. (mA)	18	18.5	19	19.5
rel. int.(%)	3	13	37	97

The formation of this ion is favoured at high temperature, even to the extent of yielding the base peak in a number of f.d. mass spectra of PC at high anode temperature.

Similarly, the ion at m/e 349 observed in compounds I, IV, V, IX, XII and VXII (Appendix I) has been assigned as dicholine pyrophosphate resulting from a condensation between a protonated molecule and a phosphoryl choline (Scheme 5).



Scheme 5. Rationalization of the Formation of Dicholine Pyrophosphate in f.d. Mass Spectra of PCs.

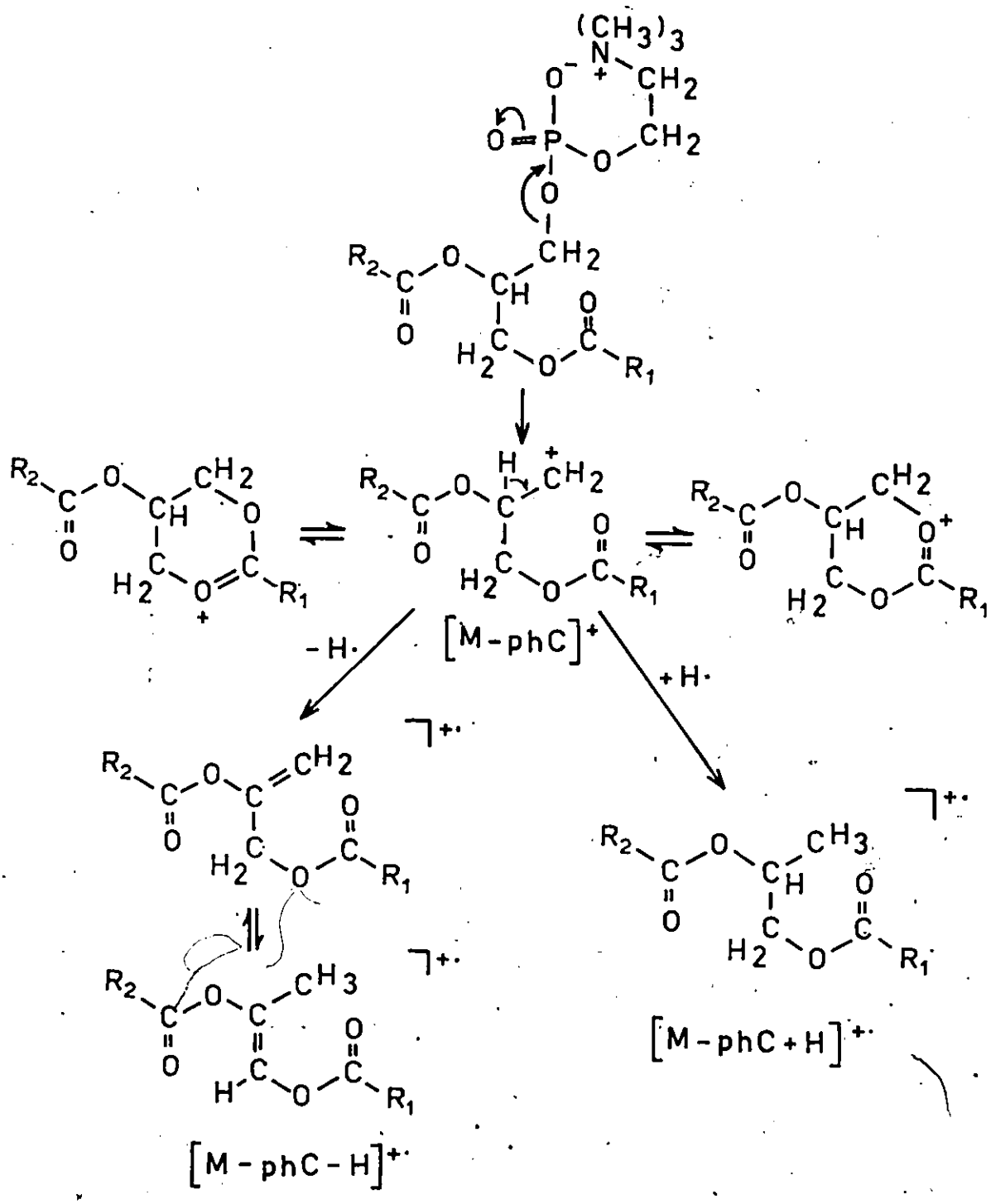
The formation pathway of m/e 198 ion corresponding to the methyl phosphoryl choline in the spectra of compounds II, V, IX, VIII, and XVII could be two-fold. Firstly, it may be formed through a direct bond cleavage between C_2-C_3 of the glycerol skeleton concomitant with hydrogen rearrangement. Alternatively, it may be attributed to the cleavage of the phosphate ester-glyceryl bond from the $M+CH_3$ ion (the formation of which will be discussed later).

A signal due to elimination of phosphoryl choline (phC) from the molecular ion (M-phC) is

also significant in most of the mass spectra listed in Appendix I. In contrast to e.i.m.s., in which this ion is the base peak regardless of probe temperature and electron energy, under f.d.m.s. conditions its relative intensity is comparatively small with respect to the protonated molecular ion at BAT. However, at higher anode temperature, the formation of this ion becomes more important. This thermally dependent process often serves as a good indicator as to whether the f.d. mass spectrum was obtained at the BAT or above. A gain or loss of hydrogen from this species was also observed in III, IV, VI, VII, IX, and XIII. (Scheme 6).

Comparison of relative intensities of some characteristic fragment ions within this category is shown in Table 3. Other peaks such as m/e 146 (5%) in I, m/e 326 (3%) in III, 124 (15%) in VII, 152 (4%) in IX, 341 (3%) in XI, m/e 188 (4%) and m/e 312-359 in XIV are not readily assigned by manipulation of the major structural units.

(2) Ions within the second category are those resulting from the cleavage of the hydrocarbon chain.



Scheme 6. Rationalization of the Formation of $[M-phC]^+$, $[M-phC-H]^+$ and $[M-phC+H]^+$ in f.d. Mass Spectra of PCs.

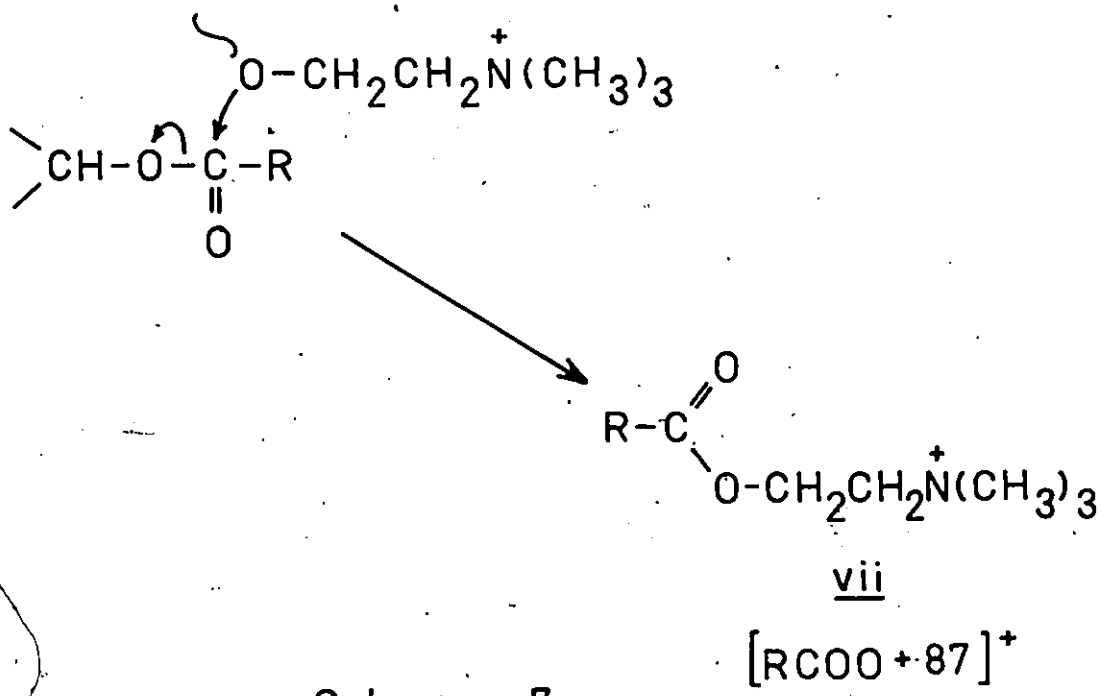
Table 3

Relative Intensities of Selective Fragments Ions of Phosphatidyl Cholines

Compounds	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII
e.h.c.(mA)[a]	18	18	21	20	19	18	23	22	21	20	19.5	20	21	19	19	21	21
m/e 86[b]	11%[c]	1%	5%	6%	12%	4%	11%	10%	45%		43%	7%	15%		8%	6%	23%
m/e 88		51	3	2	10			1	3	1%	3	5	6		2	5	9
m/e 104	7	74	3	15	53	9	8	17	47	6	22	34	68	38	252	56	46
m/e 129			31		1		15	2	8		4	3	4				5
m/e 130			4		4		3	9	9	24		2	9				5
m/e 184	2	17		4	6			2	4			3	5				2
m/e 198		2			2				4				2				2
m/e 269	13		44	5	13	4	65	8	78		98	13	10				20
m/e 349	5			4	4				4			1					6
$[M=phC-H]^+$			4			30	99		7								
$[M-phC]^+$			4			23	127	5	7		43	11	2		17		
$[M-phC+H]^+$				1									5				

[a] Emitter heating current (mA) at which the spectrum was obtained.
[b] Nominal mass number after mass defect correction.
[c] Relative intensity (%).

Among the PCs investigated so far in only a single case (compound VII) was the acylium (RCO^+) ion detected while the acyloxy (RCOO) ion and the free fatty acid were totally absent. Nevertheless, a peak corresponding to (RCOO + 87) was observed with considerable intensity (the strongest peak in f.d.m.s. of VI and VII). This ion is attributed to the choline ester of the aliphatic fatty acid (vii). It is reasonable to presume that transesterification between choline ion and the fatty acid residue facilitates the elimination of an acyl group to form this species (Scheme 7).



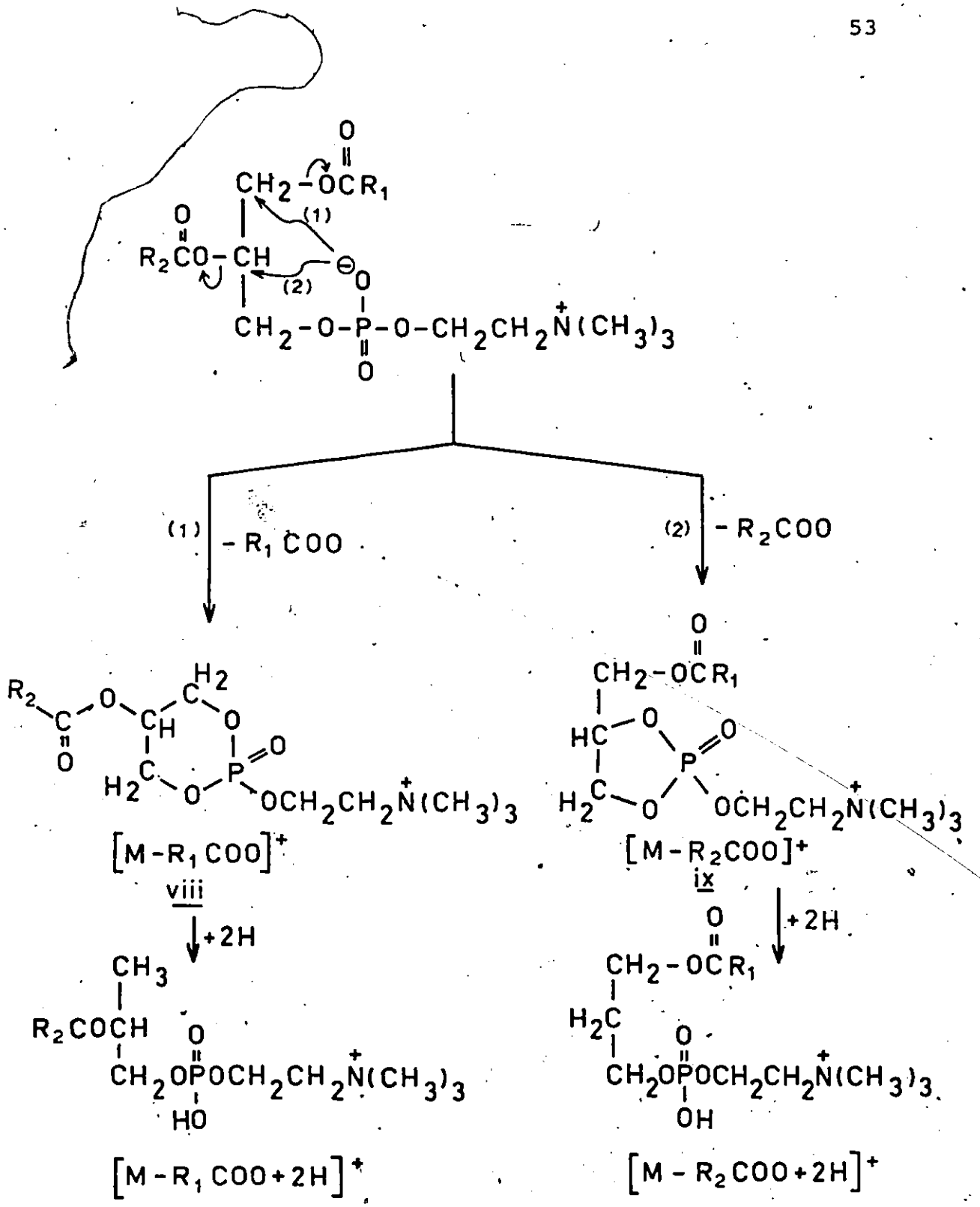
Scheme 7

Transestification Between Choline Ion and Fatty Acid Residue in f.d. Mass Spectra of PCs.

What may be the complementary charged species was registered in f.d.m.s. of compound II and XVI as $[M-RCO+2H]^+$. Since this species is equivalent to the protonated LPC, the possible contribution of impurities (as hydrolysis products of PC) to the high relative intensity of this ion (for instance, in compound II) cannot be neglected.

Formation of the $[M-RCOO]^+$ ion may involve a nucleophilic substitution (S_N) reaction (see Scheme 8) between the phosphoryl oxygen and the C-1 (pathway 1) or C-2 (pathway 2) atom of the glycerol skeleton. Loss of a fatty acid residue at the C-1 (α) position via pathway 1 would lead to 6-member ring product (viii) whereas the corresponding loss at C-2 (β) position through the latter pathway would result in the formation of a 2-3 cyclic diester bond (ix). Either cyclic structure could be destroyed by subsequent transfer of 2 hydrogens, which are shown in the Scheme 8.

Whether there may be preferential cleavage of the RCOO group at the C-1 (α) position over the C-2 (β) position is still difficult to establish at this stage. However, with reference to the data available for the five mixed fatty



Scheme 8. Rationalization of the Formation of $[M-RCOO]^+$ and $[M-RCOO+2H]^+$ Ions of f.d. Mass Spectra of PCs.

acid PCs (VII-X and XVII) it appears that the elimination of a saturated hydrocarbon chain is more favourable than that of an unsaturated one. (cf. Table 4, compound VIII, IX, X and XVII). This difference is even more pronounced when a polyunsaturated fatty acid residue is involved. A similar conclusion has been drawn[68] from the e.i. mass spectra of 1-oleoyl-2-stearoyl PC and 1-stearoyl-2-oleoyl PC.

A minor peak corresponding to $[RCO + 115]^{+\cdot}$ in compound IV (m/e 298) and V (m/e 362) may be derived from the rearrangement of (M-phC). Similar ions retaining the glyceryl moiety were obtained in many triglycerides.[123] The suggested structures are

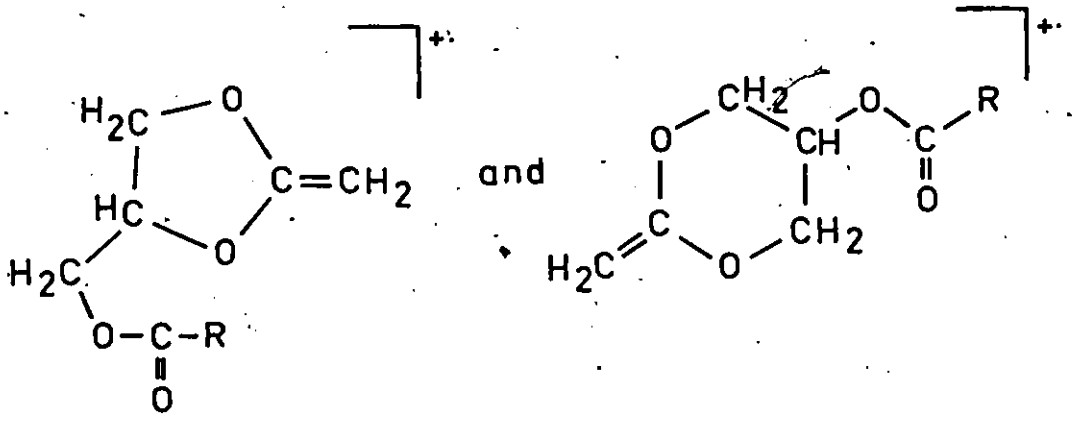
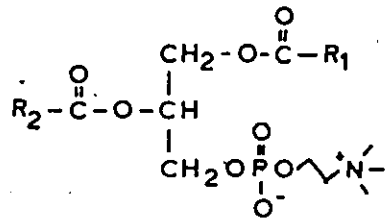
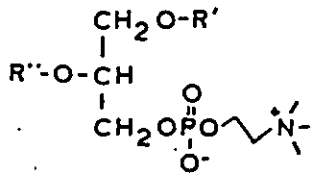


Table 4

Relative Intensities of Some Fragment Ions of Mixed Fatty Acid Phosphatidyl Cholines

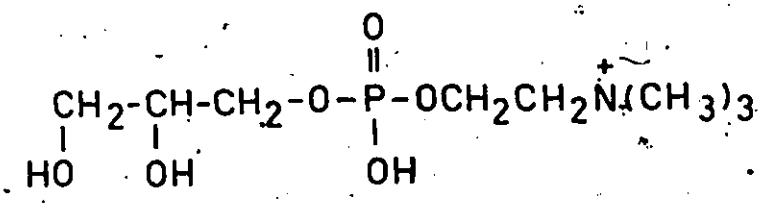




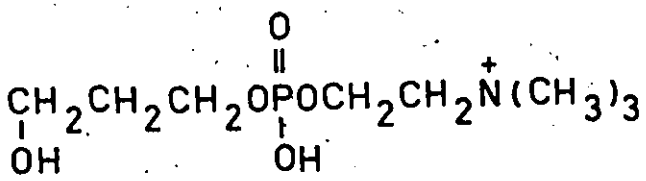
R_1COO=	*Palmitoyl	*Palmitoyl	Oleoyl	*Palmitoyl	Octadec-9-enyl[v]
R_2COO=	Myristoyl	Oleoyl	*Palmitoyl	Linoleoyl	*Hexadecyl[vi]
R_1CO	m/e 239 *(5%)[i,ii]				
R_2CO	m/e 211 (15%)[iii]				
M-R_1COO +aH	a=0, 450 *(5%)	a=1, 503[iv] *(3%)		a=0, 502 *(13%)	
M-R_2COO +bH	b=0, 418 (3%)	b=3, 481[iv] (4%)	b=0, 504 *(2%)	b=1H+Na, 562 (2%)	
M_1COO+87	m/e 342 *(8%)			m/e 342 *(78%)	
M_2COO+87	m/e 314 (5%)			m/e 366 (40%)	m/e 326[vii] *(5%)

[i] Entries in the table are of the form (m/e) followed by (rel. int.).
[ii] Ions labelled with an asterisk are resulted from the removal of a palmitoyl group.
[iii] $[CH_3(CH_2)_{14}]^+$ may have some contribution to the rel. int.
[iv] at 21 mA, m/e 503 (6%) and m/e 481 (5%).
[v] R' = octadec-9-enyl.
[vi] R" = hexadecyl.
[vii] R'O+87-H.

Simultaneous removal of two acyl groups gives rise to the peak (m/e 258, protonated glycerol phosphoryl choline, x, in compound II. Evidence for the loss of both acyl and acyloxy groups appears as a peak at m/e 242 in compound I.

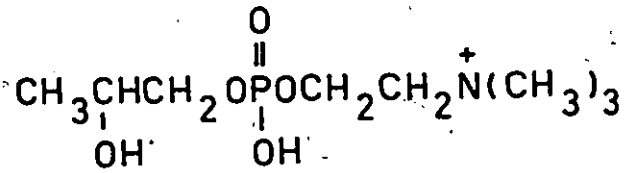


x m/e 258



xi

or



xii

m/e 242

With the exception of these two ions mentioned above, ions within this category are listed with their relative intensities in Table 5 for comparison. Part of the difference in their

Table 5

Comparison of the Relative Intensity of Some Selective Ions in f.d.m.s. of PC[a]

Compound	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII
e.h.c. (mA)	18	18	21	20	19	18[b]	23	22	21	20	19.5	20	21	19	19	21	21
RCO							239 (5)										
R_2CO							211 (15)										
M-R_1COO	m/e 310 (58)	366 (4)	394 (16)	422 (3)			450 (5)			502 (13)	507[f] (10)	504 (2)	503[f] (4)				
M-R_2COO							478 (3)		504 (2)								
M-R_1COO +2H	312 (12)	368 (41)	396 (7)	424 (8)	452 (9)		452 (3)	503[c] (3)		526[e] (2)		506 (4)	505[g] (7)				
M-R_2COO +2H							480 (4)	481[d] (4)									
M-RCO +2H	384 (34)															466 (8)	
R_1COO +87	230 (203)						342 (8)			342 (78)	371 (118)	368 (142)	367[h] (15)	364 (25)	390 (46)		326[i] (5)
R_2COO +87							314 (5)			366 (40)							
RCOO+99 (RCO+115)				298 (2)	362 (2)												

Table 5

cont'd

[a]Entries in the table are of the form: m/e (rel. int.).

[b]Owing to the low emitter heating current, no ion in this category was observed.

[c]$M-R_1COO-H$

[d]$M-R_2COO-3H$

[e]$M-R_1COO+H+Na$

[f]$M-RCOO+H$

[g]$M-RCOO+3H$

[h]$RCOO+87+H$

[i]$R_2O+87-2H$

relative intensities of the same ion depends upon operating conditions, emitter heating currents, concentrations as well as the molecular structures.

(3) Ions in the upper mass range fall into the third category. These ions involve the $[M+H]^+$ $[M+CH_3]^+$, $[M-CH_3+2H]^+$ and those ions arising from the association of a charged species with the intact molecule. Most important of all, the $[M+H]^+$ is the base peak in most of the f.d. mass spectra. Examination of isotope peaks associated with this base peak gives some assurance of the quality of these spectra. Generally, abundance of ions 1 or 2 a.m.u. above the M+H ions are in reasonable agreement with expected contribution from ^{13}C, ^{15}N and ^{18}O.

The addition of hydrogen to $[M]^+$ is due to field-induced proton transfer reactions on the emitter surface. The proton may arise from an acidic moiety of another sample molecule or from solvent molecules. The presence of a $[M-H]^+$ peak suggests that the proton transfer process may involve a bi-molecular reaction, at least in part:

$$2M \xrightarrow{-e} (M-H)^{\cdot} + [M+H]^{+} \qquad (1)$$

Secondary ionization of the resulting $(M-H)^{\cdot}$ radical left on the emitter surface may contribute to the $[M-H]^{+}$ peak of the spectrum as well as the following reaction:

$$M \xrightarrow{-e} {}^{*}H + [M-H]^{+} \qquad (2)$$

Surface reactions of these types have been discussed in serveral articles recently.[81,120,121] The high value of $[M+H]^{+}/[M]^{+\cdot}$ and $[M-H]^{+}/[M]^{+\cdot}$ ratios greater than 1 in the spectra of PCs are in agreement with the literature statement that ionization potential (i.p.) of the neutral molecule is not supplied during proton transfer reactions, and also show that the formation energy of the $[M+H]^{+}$ or $[M-H]^{+}$ according to reactions (1) and (2) respectively should be considerably less than the i.p. of the undissociated molecule. The relatively high intensity of $[M+H]^{+}$ as compared to that of $[M-H]^{+}$ (only 3-6% w.r.t. the $[M+H]^{+}$) indicates that reaction (1) is energically more favourable than reaction (2). These relationships are not only valid for protonation reactions but also for

other ion formation reactions, e.g. association reaction, cationization reactions, etc.

Intermolecular methyl transfer has been postulated[75,77] for the occurence of the peaks ($[M+CH_3]^+$ and $[M-CH_3 + 2H]^+$) flanking the molecular ion in the f.d. mass spectra of PCs. Basically, this process follows reaction (1) except that the hydrogen is replaced by a methyl group. Several experiments have been made to demonstrate that such a surface reaction occurs in the condensed phase on the field anode. Firstly, choline chloride (XVIII), choline bromide (XXI) as well as choline(methyl-d_9) chloride (XVIII) were chosen as simpler systems to study the mechanism of this intermolecular transfer. Their f.d. mass spectra are shown in Figs. 3, 4 and 5. As reported earlier[78] the first three compounds containing a free hydroxyl all show a peak at m/e 118 $(M+14)^{\dagger}$ as well as a peak at m/e 89 (M-15). However, if the hydroxyl hydrogen was substituted by an acetyl group, neither of these ions was seen (Fig. 4). When a choline halide was dissolved in D_2O, about one half of its hydroxyl hydrogen was exchanged as

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†The cation of the onium compound will be referred to as the molecular ion.

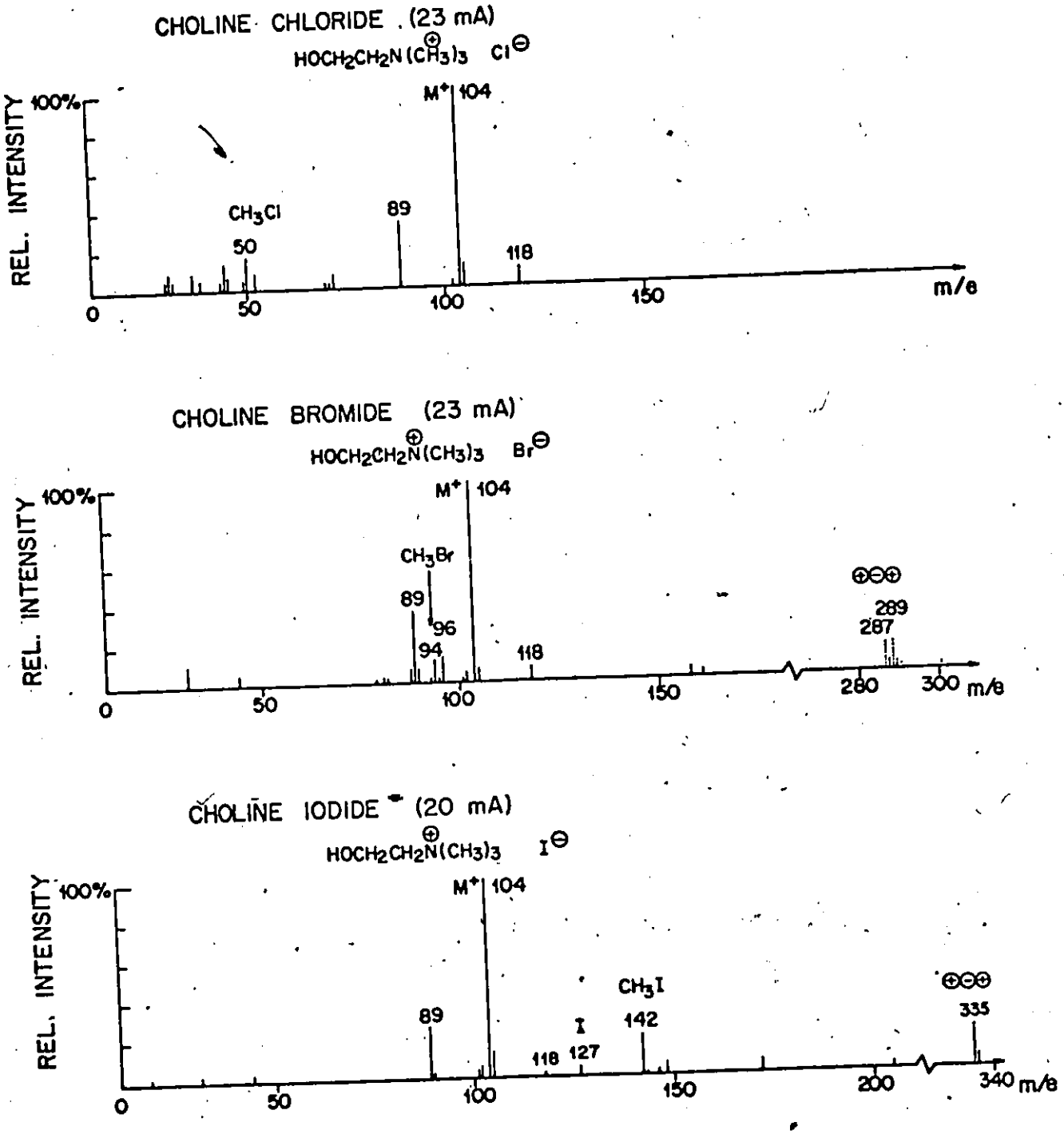


Fig. 3. F.d.m.s. of choline chloride (XVII), bromide (XIX) and iodide (XX). Broken lines at m/e 287, 289 for the bromide indicate that this ion cluster was not recorded on every scan.

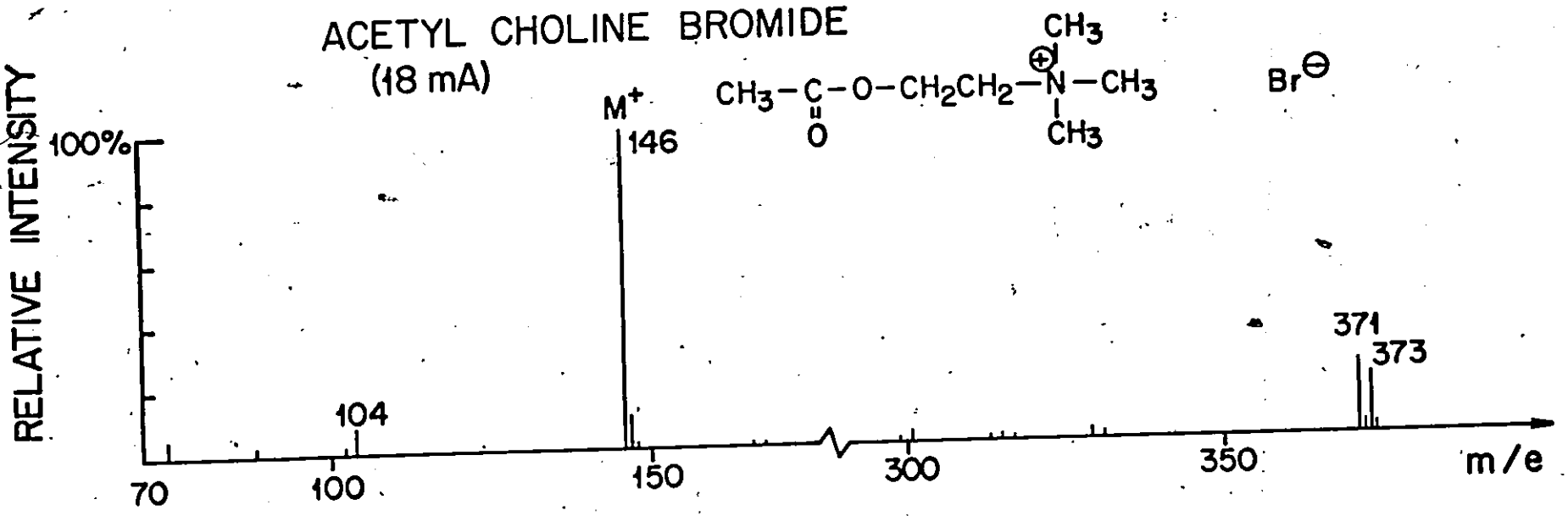


Fig. 4. Field Desorption Mass Spectrum of Acetylcholine Bromide (XXI).

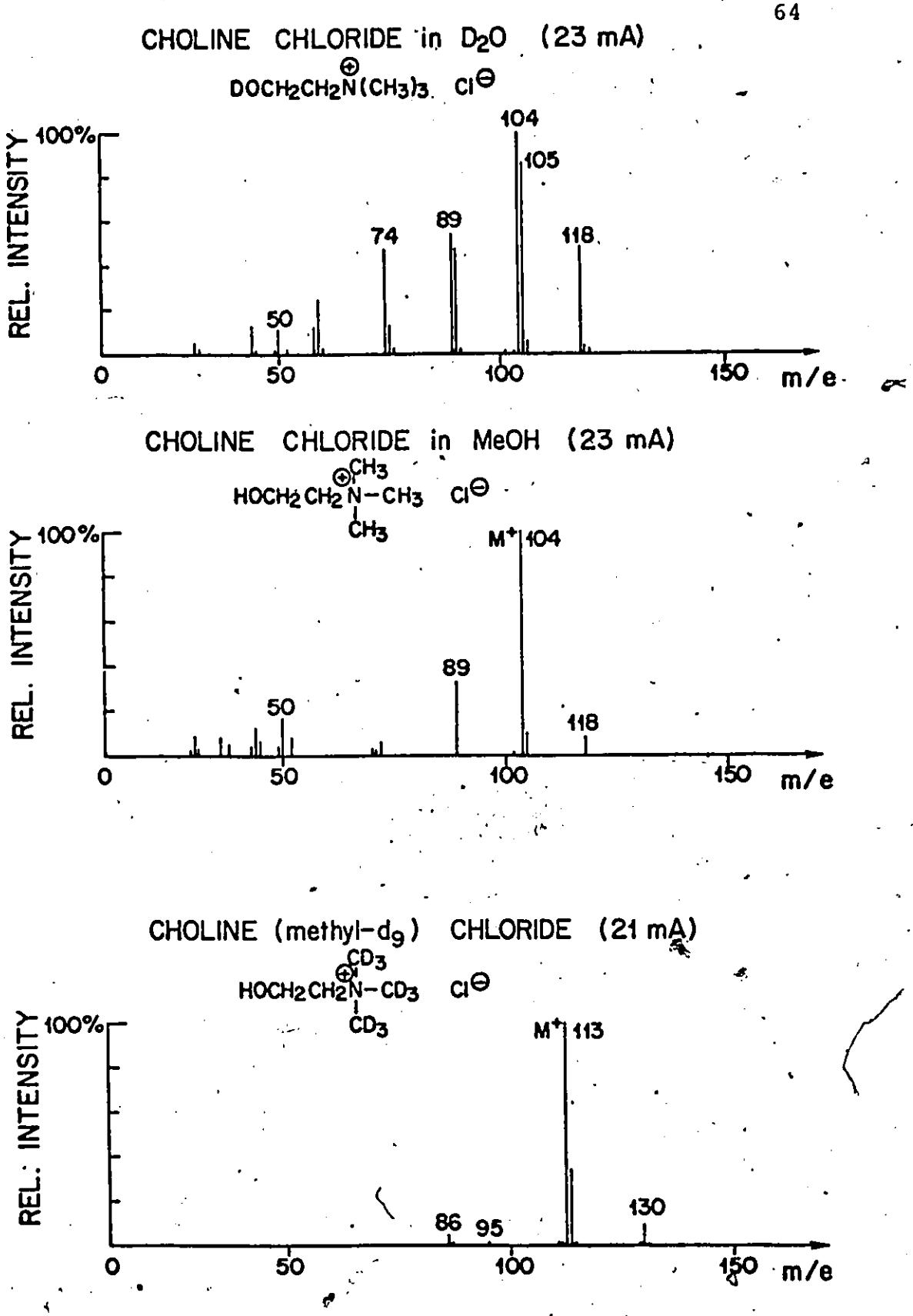
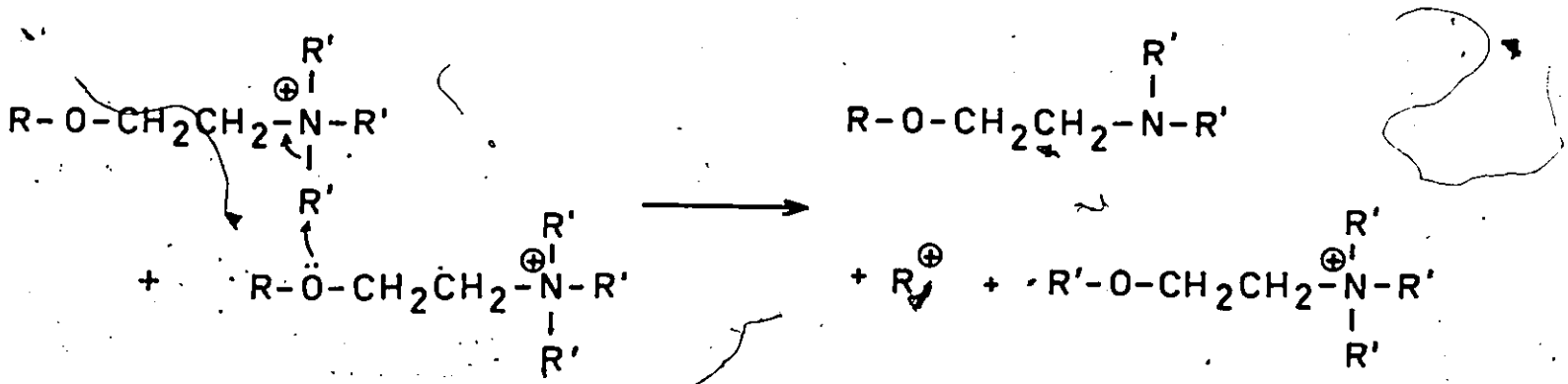


Fig. 5. Field desorption mass spectra of choline chloride in D_2O (a) and MeOH (b) and labelled-d_9 analog (c).

indicated by m/e 104 and 105 peaks with almost equal intensity (Fig. 5 (a)). A doublet of almost the same ratio as m/e (104/105) was also observed at m/e 89 and 90. Nevertheless, only a single peak at m/e 118 (and none at m/e 119) was detected. It is difficult to escape from the conclusion that the exchangable hydrogen (deuterium) must be lost during the intermolecular transfer process. The spectrum obtained from choline (methyl-d_9) chloride is presented in Fig. 5(c), as compared with the mass spectrum of the unlabelled compound. The molecular ion of the d_9- compound is now shifted to m/e 113. Significantly, the ion at m/e 130 now corresponds to $[M+17]^+$. Thus, the simplest explanation for this ion is the transfer of a methyl group from ammonium nitrogen to another molecule. The mechanism proposed for this exchange process is shown in Scheme 9, and the results are summarized in Table 6. Further evidence was provided by the presence of $(M-CH_3)$ and $(M+CH_3-H)$ ions in the f.d.m.s. of phosphoryl choline chloride (see Section H). A mechanism of this kind can also be applied to the present case of PC. The fact that the M+15 peak in VI-d_o and VI-d_4 becomes M+18 $(M+CD_3)$ for VI-d_9 provides clear evidence that



$$2\ M \longrightarrow R + (M - R') + \left[(M - R) + R'\right]$$

Scheme 9. Rationalization of Intermolecular Methyl Transfer in Choline Halides.

Table 6.

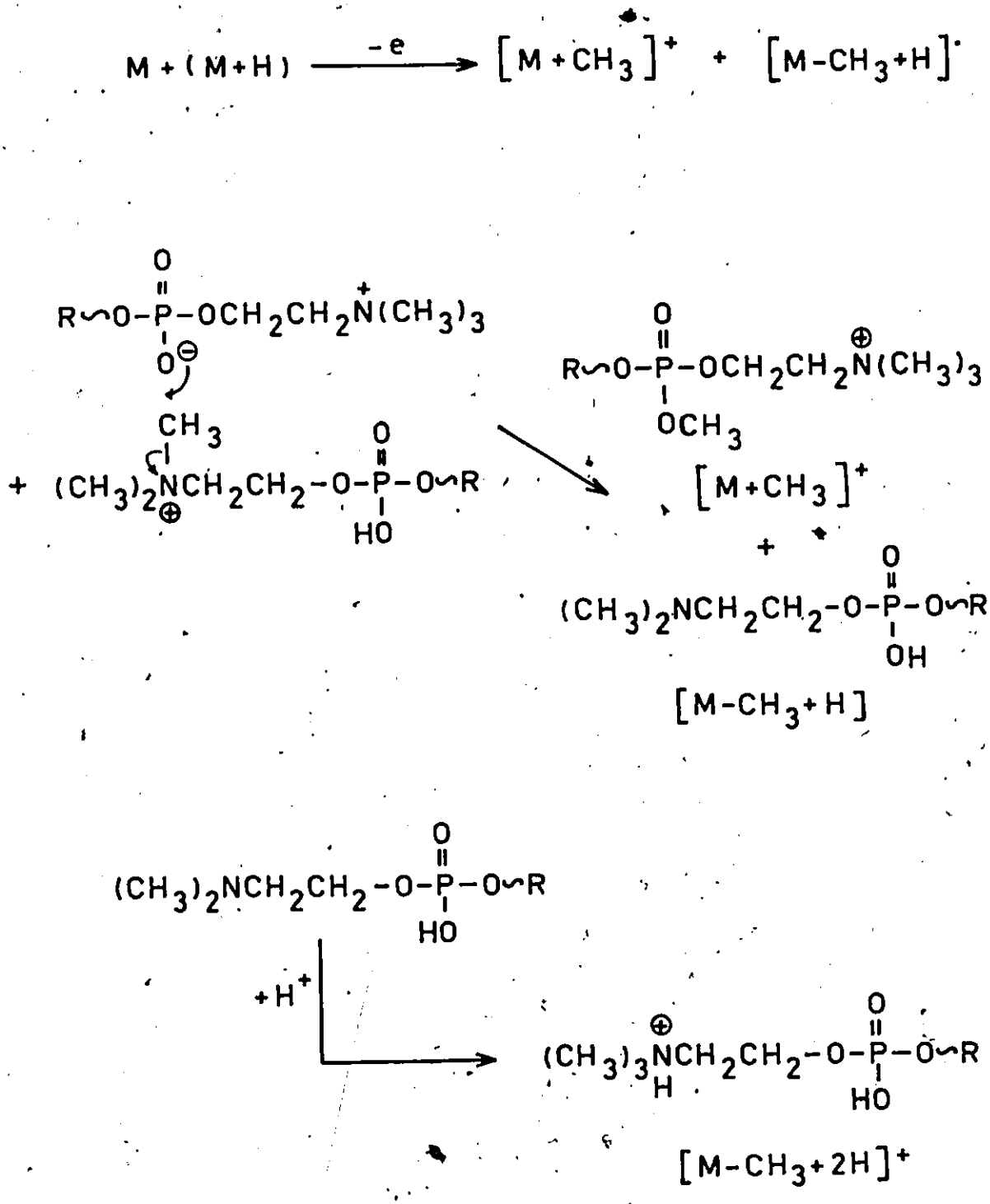
Summary of f.d. Mass Spectra of Choline Halides in MeOH and D_2O, Acetylcholine Bromide and d_9-Choline Chloride.

R	R'	$M^{+\cdot}$ (m/e)	$[M-R']^+$	$[M-R+R']^+$
H—	CH_3—	104	89	118
D—	CH_3—	105	90	118
CH_3-C(=O)—	CH_3—	146	(131)*	(118)*
H—	CD_3—	113	96	130

*Ions within the parentheses were not recorded

the intermolecular methyl transfer does indeed occur in PC and the methyl group of the choline moiety should be responsible for such a transfer reaction. Although the terminus for this intermolecular transfer is not so easy to establish, the phosphate group seems to be a good candidate. Accordingly, the postulated mechanism (as shown in Scheme 10) for the generation of $M+CH_3$ ion involves a nucleophilic attack of the phosphoryl oxygen on the ammonium methyl group of another protonated molecule. Secondary protonation required for the ionization of the other complementary species (phosphatidyl N,N-dimethylethanolamine) may account for the relatively low intensity of the $[M-CH_3+2H]^+$.

Cationization of the intact molecule by ubiquitous sodium and potassium ions, a prevailing feature of f.d.m.s. of polar compounds, gives rise to the $[M+Na]^+$ and $[M+K]^+$ peaks. The bonding of a cation to a molecule is less chemical in nature than, for instance, the attachment of a proton or alkyl ion, but is principally controlled by coulomb and polarization forces. These alkali metal ions, as impurities, are presumably incorporated during synthetic and/or chromatographic procedures. The possibility of ionization of



Scheme 10. Rationalization of Intermolecular Methyl Transfer in f.d. Mass Spectra of PCs.

polar molecules by alkali ion attachment at the surface of a field ion emitter has recently been demonstrated by Röllgen and Schulten.[124,125] In f.d.m.s. of organic molecules, this process is particularly important from an analytical point of view, since the addition of an alkali metal cation to a large organic molecule containing nucleophilic groups produces stable even-electron ions, whereby the possibility of ionization and of detection of the intact molecule is increased. Furthermore, the presence of $[M+Na]^+$ or $[M+K]^+$ ions allow a decision to be made between $[M]^{+\cdot}$ or $[M+H]^+$ ions if they are also recorded. The greater stability of the cationized molecules than that of the corresponding $[M]^{+\cdot}$ and $[M+H]^+$ ion may be attributed not only to the lower recombination energy (ca. the ionization potential (i.p.) of the alkali atoms) compared to the i.p. of the molecule, but also to the enhanced activation energy required for fragmentation because of the necessity of achieving charge shift in the cationized complex.[124] Among the alkali ions and other cations, lithium has the largest affinity for polar molecules.[84] For a given cation, the stability of the complex roughly depends on the magnitude of the dipole moment of

the functional group in the molecule being investigated.

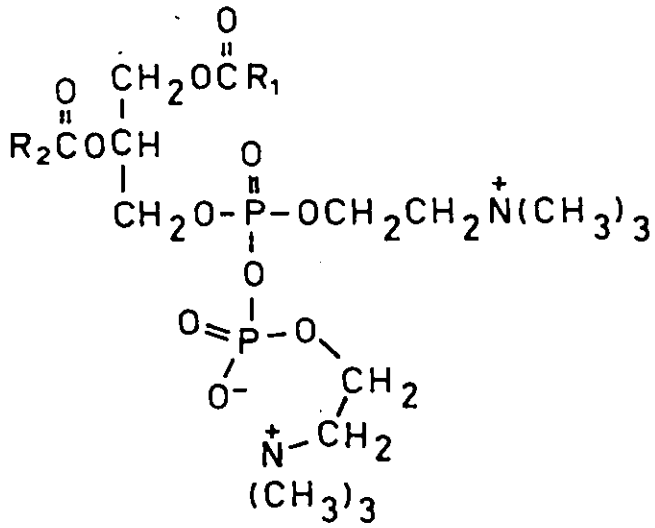
However, as mentioned in the previous chapter, the synergistic effect of cationization can be easily destroyed when larger quantities of inorganic salts are present in the sample. The stronger binding of the organic substance in the lattice of the inorganic salt requires much higher anode heating for f.d. and under these circumstances the molecules will then decompose thermally before molecular ion formation or cationization can take place. In such cases, it is necessary to remove the inorganic salts from the sample by suitable physical or chemical methods (as mentioned in the previous chapter) before the f.d.m.s. analysis.

Ion association reaction between the intact molecule and one of the charged species as represented in Scheme 1 is a striking feature of f.d.m.s. of PC. The nature of the association is not fully understood but it is believed that it may involve an ionic interaction in an analogous manner to the $\{M+\text{alkali ion}\}^+$. The phosphoryl oxygen seems to be an ideal site for such an ion-molecule interaction to take place. Again, because of the low recombination energy

for such an association reaction, the resulting association ion clusters are then stabilized (as compared to the radical molecular ions or even the protonated molecules) as long as the positive charge remains localized at the attached organic cation. Thus the charge shift by arrangement of the bonding electrons within the ion necessary for a decomposition may be prevented.[124] In associated ion clusters, the process with the lowest activation energy is usually the splitting off of the cation rather than the fragmentation of the molecule.[124]

Associated ion clusters with mass number higher than that of the molecular ion together with the proposed structure of the charged species are shown in Table 7. Correct mass assignment of these ions with such a high m/e value (m/e 700 or above) is difficult to achieve without using a suitable mass marker in this mass range. The routinely used chemical mass marker perfluoro-kerosene (PFK) is not useful for mass measurement above m/e 750. Although other new reference standards with much higher molecular weight (e.g. perfluorotriheptyltriazine mol. wt. = 1184, triperfluorotrinonyltriazine mol. wt. = 1485) have been recently reported.[126,127] Our

Table 7. Associated Ion Clusters in f.d. Mass Spectra of PCs.

M + 23	M + Na^+
M + 39	M + K^+
M + 58	M + $CH_2=\overset{+}{N}(CH_3)_2$
M + 72	M + $CH_2=CH\overset{+}{\underset{H}{N}}(CH_3)_2$
M + 86	M + $CH_2=CH\overset{+}{N}(CH_3)_3$
M + 104	M + $HOCH_2CH_2\overset{+}{N}(CH_3)_3$
M + 184	M + PHOSPHORYL CHOLINE
M + 269	M + DICHOLINE PHOSPHATE
M + 166	
2M + H	

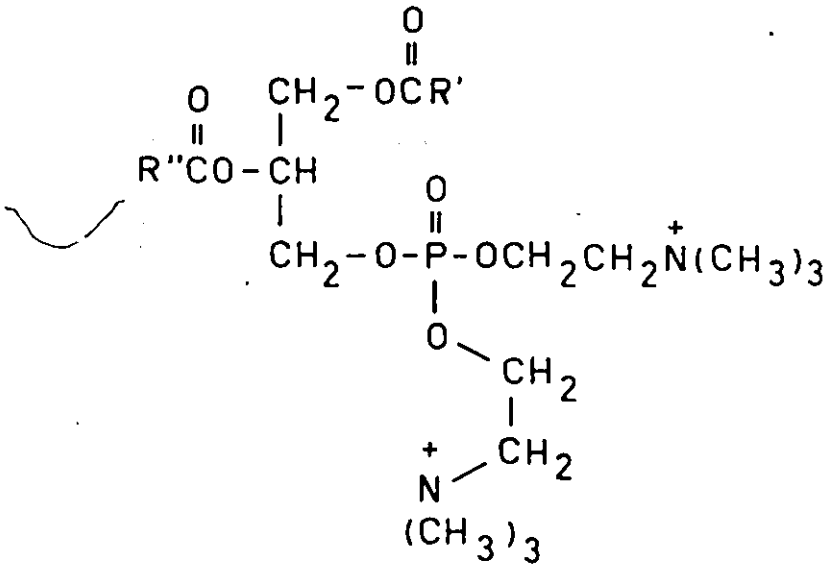
experience suggests that they have a destructive effect on the cathode under normal e.i. operation. For this reason, they are not used in this laboratory. Another approach by reducing the normal accelerating voltage of 3kV to 2kV is also feasible to determine the masses ions at high m/e. In this way the mass marker is "stretched" by a factor of 3/2 so that the calibration scale is extended from 750 to 1125. However, this technique suffers considerable loss of sensitivity of ion detection. This is important especially when only a small number of ions are generated. The mass assignments reported in Table 7 rely on the extrapolation of the mass scale above m/e 750, thus an error of one or occasionally two a.m.u. higher or lower than the actual mass number of the ion is expected.

The ion corresponding to (M+166) as shown in Table 7 does not appear to be an associated ion cluster, instead, it may represent 1,2-diacyl-sn-glycero-3-bis-phosphorylcholine (structure xiii) formed via condensation from a phosphoryl choline to the intact molecule in a similar manner to the formation of dicholine pyrophosphate (m/e 349, p. 47).

Some additional characteristic ions were observed in f.d. mass spectra of PCs containing short acyl chain they are:

(a) hydrated (M+166) ion for compound I: $(M+166+H_2O)$

(b) doubly charged ion $[M+87]^{2+}$ (xiv) observed in compounds I, III and IV.



xiv

$[M+87]^{++}$

(c) $[2M+H]^{+}$ ion

(d) $[2M+H+87]^{2+}$ ion as observed in compound I.

Table 8 presents a comparison of the relative intensities of some characteristics ions at the upper mass range.

Table 8-A

Relative Intensities of Some Ion Clusters in f.d. Mass Spectra of Phosphatidyl Cholines[a,b]

Compound	I	II	III	IV	V	VI	VII	VIII
M-2CH$_3$+2H					650 (4)			
M-CH$_3$+H							692[c] (8)	746 (6)
M-H		508 (6)		620 (3)	676 (3)			758 (6)
M+H	m/e 398 (100%)	510 (100)	566 (100)	622 (100)	678 (100)		706 (100)	760 (100)
M+CH$_3$	412 (1)		580 (13)	637 (3)	692 (4)		721 (8)	774 (12)
M+Na			588 (19)		700 (8)		728 (8)	782 (33)
M+29								
M+45			610 (8)		722 (3)		761[d] (15)	
M+58					735 (2)		763 (5)	
M+72					749 (3)			831 (3)
M+86	483 (3)		651 (26)	707 (3)	763 (4)		791 (5)	845 (6)
M+88		598 (3)			765 (3)			847 (3)
M+104	501 (6)	613 (7)	669 (28)	725 (6)	781 (13)		809 (5)	863 (15)
M+127								888 (3)
M+130								889 (4)
M+166	563 (17)			787 (1)				925 (2)
M+184	581 (5)	693 (6)		805 (5)	861 (5)			943 (2)
M+198								956 (3)

...cont'd

Table 8-A (cont'd)

Compound	I	II	III	IV	V	VI	VII	VIII
M+230		740 (8)						
M+269	266 (3)				946 (5)			
2M+H	795 (33)							
$[M+87]^{2+}$	242 (8)		326 (3)	354 (2)				
$[2M+87+H]^{2+}$	441 (4)							
other ions	M+312 (8)	M+ RCOO +87 (8)						
		M-RO+2H (3)						

[a] Entries are in the form of m/e, (relative intensity).

[bb] All masses indicated are nominal masses and corrected for mass defect.

[c] $[M-CH_3+H]$

[d] [M+56]

[e] [M+44]

[f] [M+57]

Table 8-B

Relative Intensities of Some Ion Clusters in f.d. Mass Spectra of Phosphatidyl Cholines[a,b]

Compound	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII
$M-2CH_3+2H$									
$M-CH_3+H$	746 (2)		775[c] (18), $M+H-CH_3$	772 (5)				691[c] (14)	718[d] (9)
M−H	758 (4)			784 (4)					
M+H	760 (100)		790 (100)	786 (100)	782 (100)	778 (100)	830 (4)	706 (100)	732 (100)
$M+CH_3$	774 (17)		804 (26)	800 (5)	796 (5)			720 (6)	746 (9)
M+Na	782 (8)	780 (100)		808 (16)	804 (86)	800 (16)	852 (22)	728 (13)	
M+29								734 (13)	760 (5)
M+45			833[e] (3)						
M+58	816[f] (4)		847 (8)	843 (2)				763 (9)	789 (3)
M+72	831 (8)		861 (7)	857 (3)					803 (3)
M+86	845 (12)		875 (18)	871 (4)	867 (3)			791 (5)	817 (4)
M+88			877 (5)	873 (3)				793 (6)	
M+104	863 (19)	861 (1)	893 (28)	889 (11)	885 (15)	881 (65)	933? (100%)	809 (16)	835 (7)
M+129			916? (7)		910? (2)				
M+130			917? (5)		911? (2)				
M+166	923? (6)		955? (11)						
M+184	944? (3)		986? (15)						929? (1)

... cont'd

Table 8-B

cont'd

Compound	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII
M+230									
M+269									
2M+H									
$[M+87]^{2+}$									
$[2M+87+H]^{2+}$									
other ions									

[a] Entries are in the form of m/e (relative intensity).

[b] All masses indicated are norminal masses and corrected for mass defect.

[c] $[M-CH_3+H]$

[d] [M+56]

[e] [M+44]

[f] [M+57]

B. Lysophosphatidyl Cholines (LPC)†

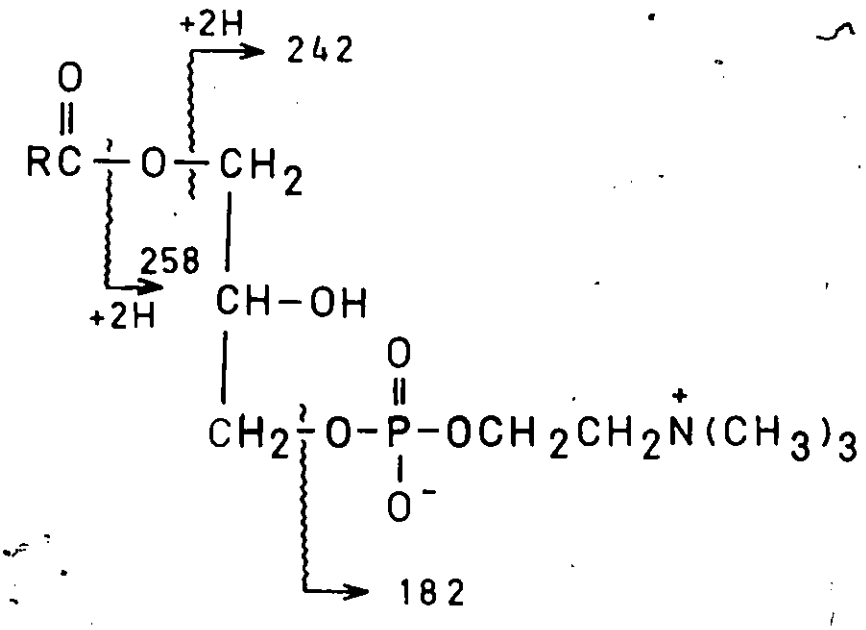


Chart 2

Cpd.	Name		M.W.
XXII	Palmitoyl LPC	$R=(CH_2)_{14}CH_3$	495.3
XXIII	Stearoyl LPC	$R=(CH_2)_{16}CH_3$	523.4
XXIV	Oleoyl LPC	$R=(CH_2)_7CH=CH(CH_2)_7CH_3$	521.4
XXV	Linolenoyl LPC	$R=(CH_2)_4(CH=CHCH_2)_3(CH_2)_3CH_3$	517.4
XXVI	Arachidonyl LPC	$R=(CH_2)_3(CH=CHCH_2)_4(CH_2)_3CH_3$	543.4

†Structure shown below places acyl group at C-1 position, but it may be present at C-2. This uncertainty precludes any conclusion about the influence of acyl group position on the spectrum.

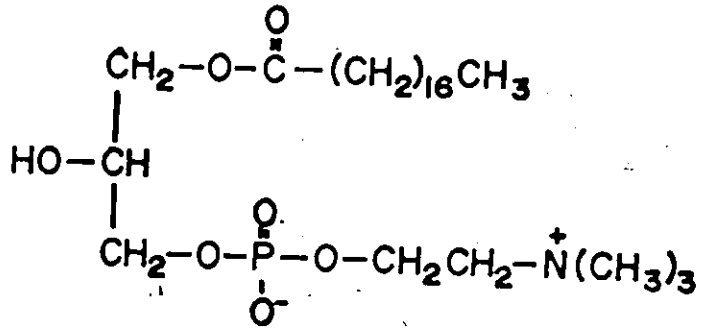
Five synthetic lysophosphatidyl cholines, namely palmitoyl LPC (XXII), stearoyl LPC (XXIII), oleoyl LPC (XXIC), linolenoyl LPC (XXV) and arachidonyl LPC (XXVI), behave in a similar manner to the PCs, under f.d.m.s. conditions. Their mass spectra are listed in Appendix II. As an illustrative example, the mass spectrum of compound XXIII is presented in Fig. 5.

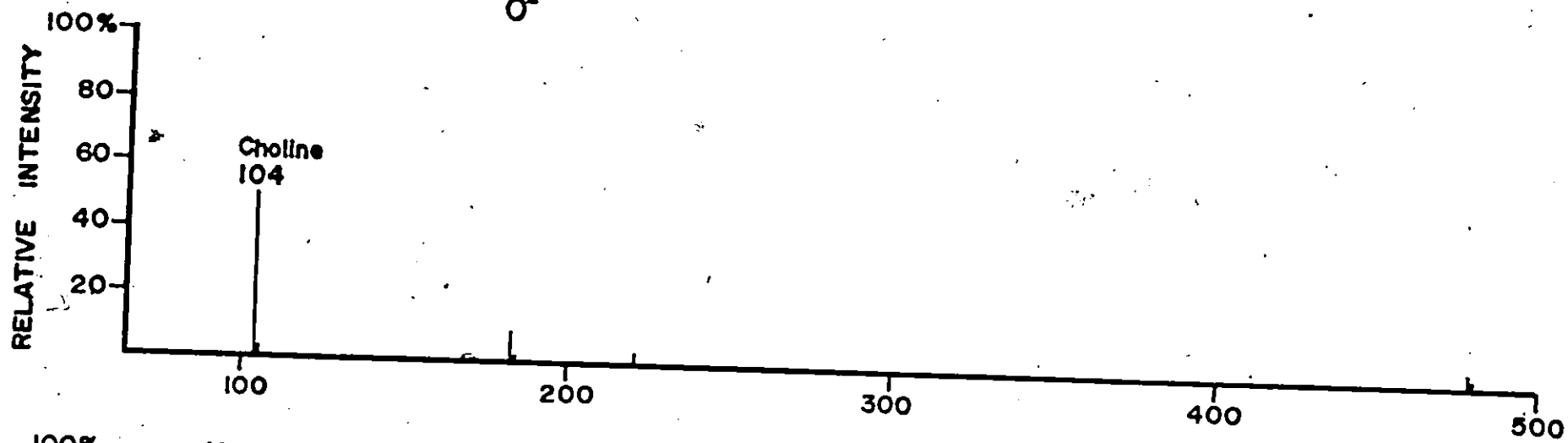
All five of these compounds yielded mass spectra which exhibited M+H ion as the base peak. Apart from those ions formed in an analogous way to those of PC, several fragments characteristic to the lyso-group were found. Ions at m/e 242 and 258 are derived from the loss of acyloxy and acyl group respectively. Structural information about the fatty acid residue can be derived accordingly.

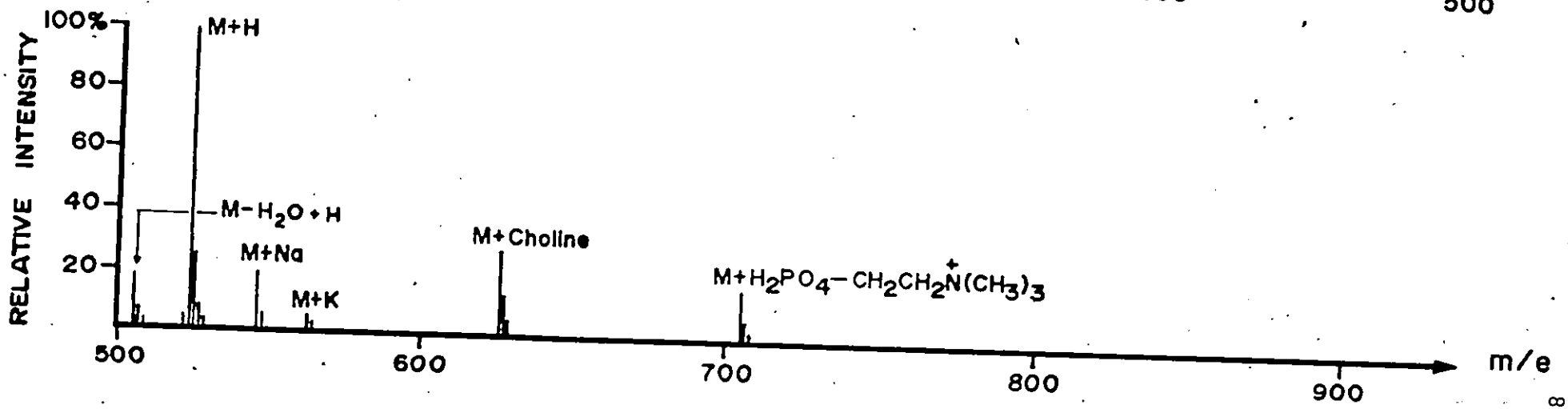
In the presence of sodium ions, cationization of phosphoryl choline (m/e 206, 184-H + Na), glycerophosphoryl choline (m/e 280, 258-H + Na) were significant as was the molecular ion. Apparently because of the presence of a free hydroxyl group in the glyceryl moiety, dehydration of the protonated molecule giving rise to the ion $[M+H-H_2O]^+$ is another important feature of LPC. An associated ion cluster, in addition to those reported in the case of PC, corresponding to the molecular ion complex with a protonated glycerophosphoryl choline (M+258) is a fairly abundant peak in XXIV (13%) and XXVI (5%).

Fig. 6 . Field Desorption Mass Spectrum of Stearoyl Lysophosphatidyl Choline.

FDMS OF STEAROYL LYSOLECITHIN (18mA) M.Wt. 523







It is interesting to notice that except for XXIV, intermolecular methyl transfer leading to the $[M+CH_3]^+$ and $[M-CH_3+2H]^+$ ions is not favorable in this case. Whether this result should be considered as a general feature of this class of compound or as a result of low e.h.c. being used is not clear, perhaps it may also be attributed to the different orientation of the molecules in the condensed phase on the emitter surface. Further studies along this line should be made before a specific conclusion can be drawn.

C. Phosphatidyl Ethanolamines (PE):

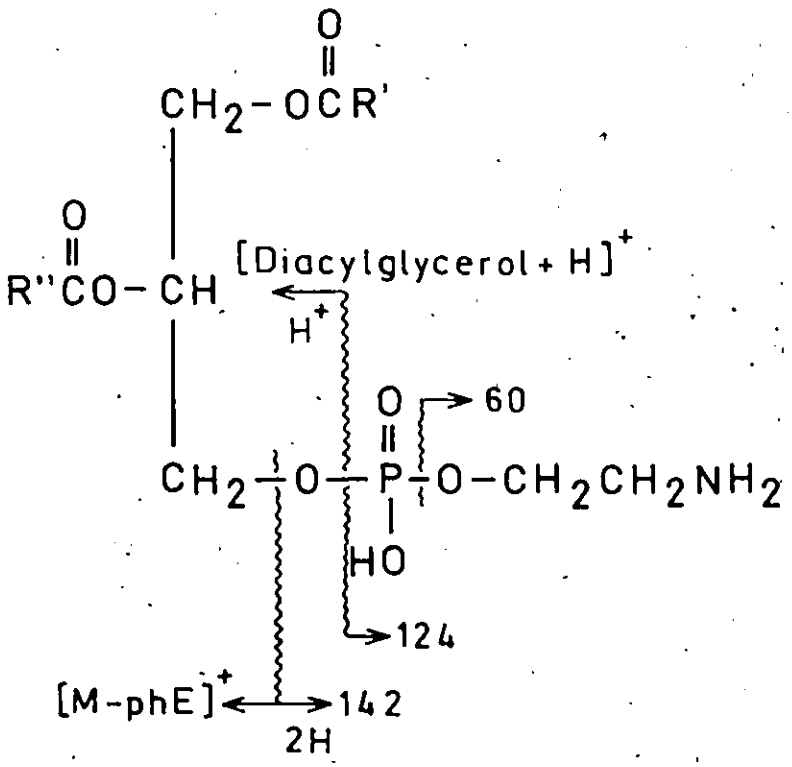


Chart 3

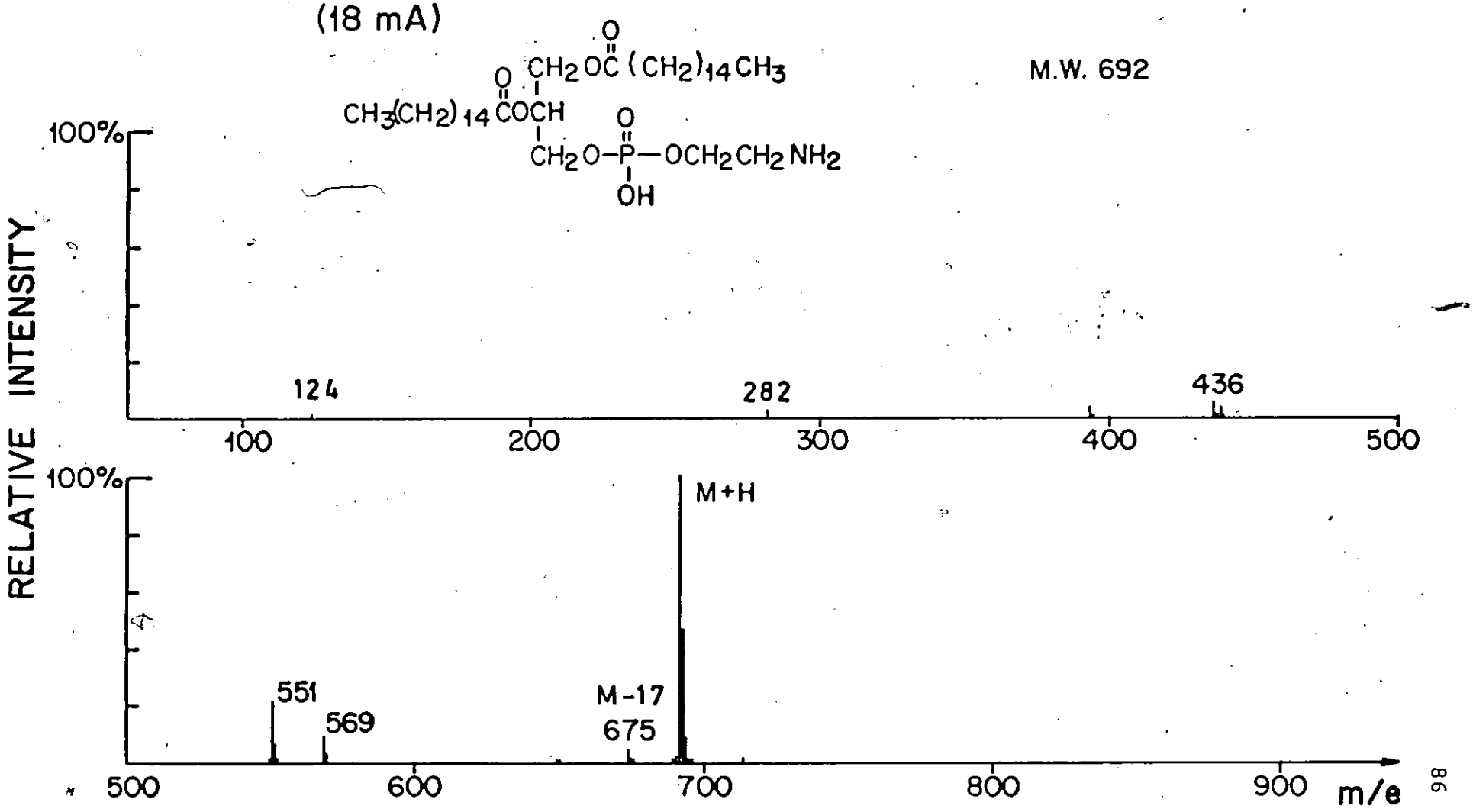
Cpd.	Name	Fatty Acids	M.W.
XXVII	Dilauroyl PE	R'=R"=$CH_3(CH_2)_{10}CO$	579.4
XXVIII	Dipalmitoyl PE	R'=R"=$CH_3(CH_2)_{14}CO$	691.5
XXIX	Distearoyl PE	R'=R"=$CH_3(CH_2)_{16}CO$	747.6
XXX	Dioleoyl PE	R'=R"=$CH_3(CH_2)_7CH=(CH_2)_7CO$	743.6

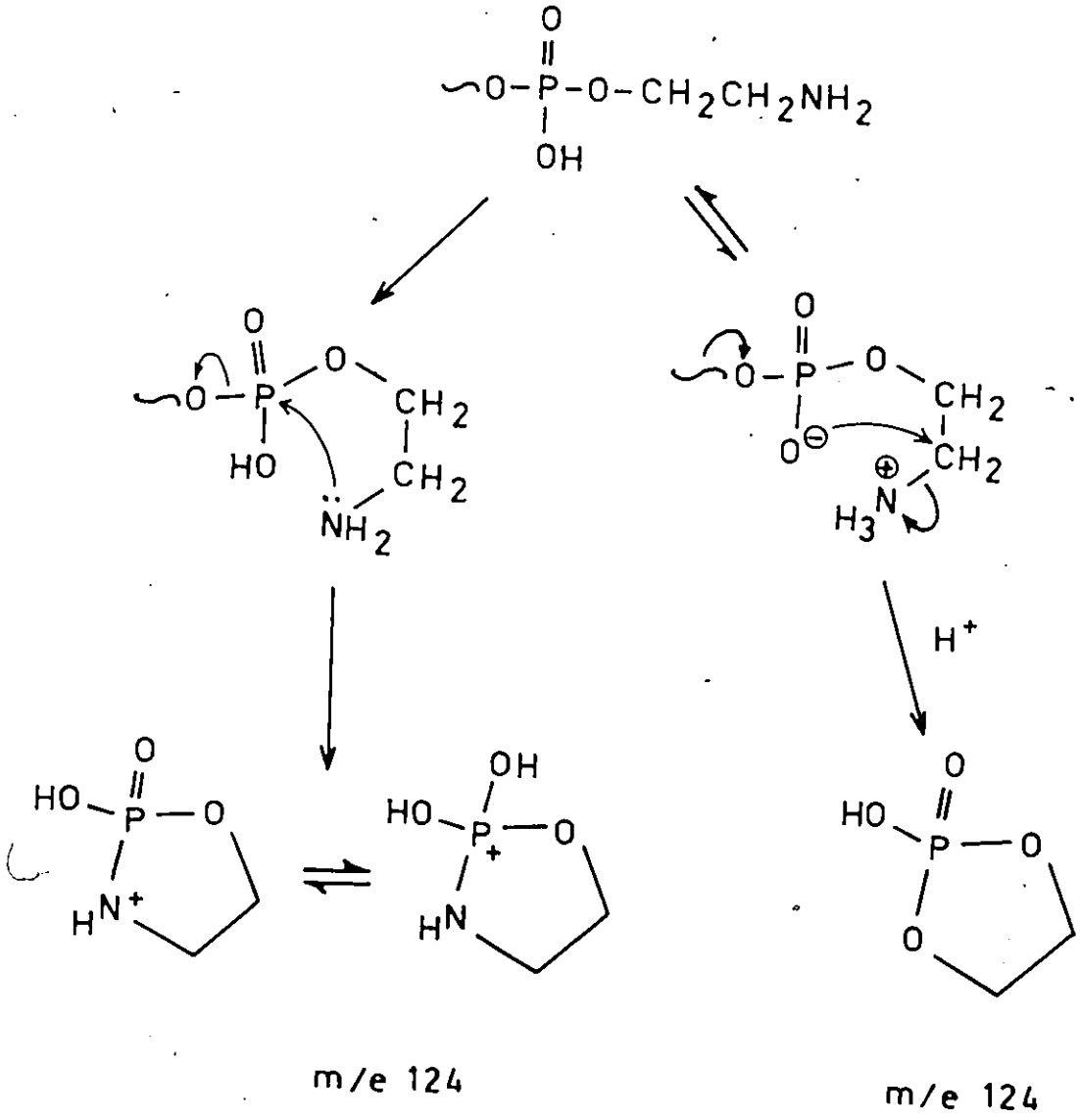
Phosphatidyl ethanolamines (PE) are another class of phospholipids not amenable to e.i.m.s. unless a TMS derivative is formed.[62,63,67] On the other hand, f.d. mass spectra of a series of PEs (XXVII-XXX) all displayed a M+H ion as the base peak making identification of the compound relatively easy. Our results (Appendix III) are also in agreement with the data from another laboratory.[76] Comparatively speaking, the f.d. mass spectra of PEs (e.g. Fig. 7) contain less fragment ions than those of the corresponding PCs, perhaps in part because of the lower BAT.

An ion at m/e 124 may be formed via two similar routes as shown in Scheme 11.

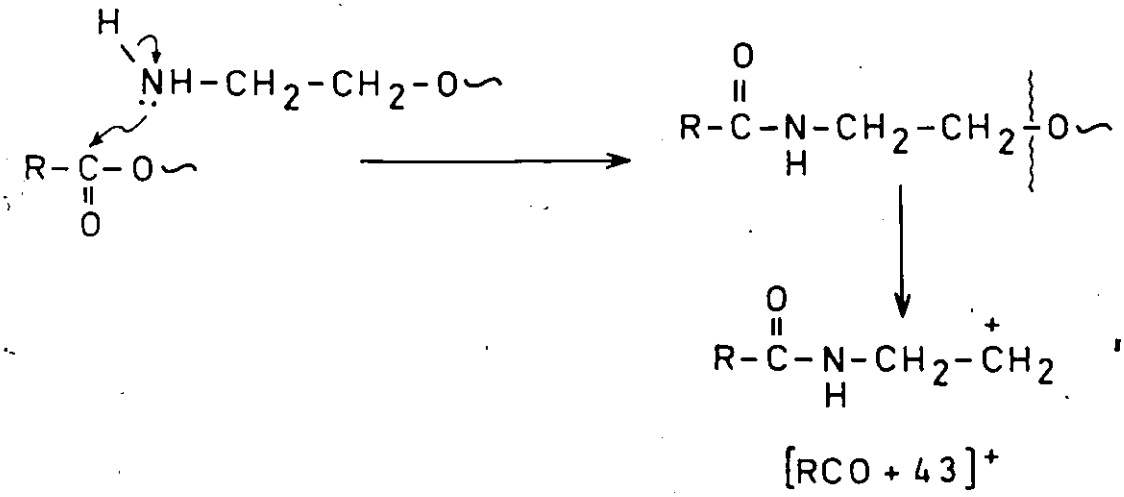
As shown in Scheme 12, an interaction between the free amino group and the acyl group could be the main derivation of the ion (RCO+43) (m/e 226, 282, 310 and 308 in XXVII, XXVIII, XXIX and XXX respectively).

FIG. 7 FDMS OF DIPALMITOYL-PHOSPHATIDYLETHANOLAMINE





Scheme 11. Rationalization of the Formation of m/e 124 Ion in f.d. Mass Spectra of Phosphatidyl Ethanolamines.



Scheme 12. Rationalization of the Formation of [RCO+43] Ion in f.d. Mass Spectra of PEs.

The possibility of losing a fatty acid residue from the neutral molecule is dependent on the chain length and perhaps on the degree of unsaturation of the fatty acids. The relative intensity of the (M-RCOO) ion increases from 4% in dilauroyl $(C_{12:0})^{\dagger}$ PE up to 22% in distearoyl $(C_{18:0})$ PE and slightly decreases to 16% in the case of dioleoyl $(C_{18:1})$ PE. Similarly, ions corresponding to [M-phosphoryl ethanolamine]$^+$ and [1,2-diacyl glycerol + H]$^+$ follow the same trend (cf. Table 9).

$^{\dagger}C_{12:0}$ designates the acyl group containing 12 carbon atoms and 0 C=C bond.

Table 9

Relative Intensities of Some Characteristic Fragments in f.d.m.s. of PEs

Compound	XXVII	XXVIII	XXIX	XXX
R	$C_{12:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$
e.h.c.[a]	18 mA	18 mA	18 mA	19 mA
$[RCO+43]^+$	m/e 226[b] (3%)[c]	m/e 282 (3)	310 (18)	308 (6)
$[M-RCOO]^+$	380 (4)	436 (6)	464 (22)	462 (16)
$[M-RCOO+2H]^+$	382 (6)	438 (4)	466 (9)	464 (10)
$[M-phE]^+$	439 (4)	551 (12)	607 (45)	603 (6)
$[diacyl\ glycerol + H]^+$	457 (3)	568 (8)	625 (12)	621 (11)

[a]Anode heating current at which the spectrum was obtained.

[b]Nominal mass number after mass defect correction.

[c]Relative intensity.

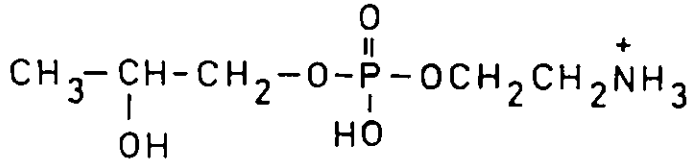
Elimination of small, stable molecules from the molecular ion or M+H is another prevailing feature of f.d.m.s. This has been observed in amino acids (H_2O and NH_3),[128] peptides (CO_2),[129-130], sugars (H_2O)[132] chlorinated hydrocarbons (HCl)[133] and sulphonic acids (H_2SO_3).[84]

It is interesting to notice that, except the $[M+Na]^+$ ion, associated ion clusters in the upper mass range are largely absent. This fact reveals that the ion-molecule association reaction, as observed in choline containing phospholipids, is more favourable for those zwitterionic than neutral compounds. In the present case, since PEs exit as neutral molecules (as shown in chart III) in solid state or in organic solvents, the site (phosphoryl oxygen) where the association is believed to be taking place is already occupied by a hydroxyl hydrogen, making the formation of an ion pair less feasible. Different extent of polar head interaction between adjacent molecules perhaps is another reason for this difference. Consideration of the BAT, or more practically the melting point of different phospholipids, can provide some impression about the intermolecular interactions existing in the crystal structure of these phospholipids. It is expected that the stronger the base, the stronger the interaction and this is indeed found in order of decreasing basicity PC > PE > PS, the same order as decreasing BAT as well as melting point.[118]

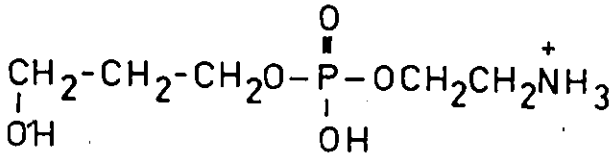
D. Lysophosphatidyl Ethanolamine (LPE):

F.d. mass spectra of palmitoyl LPE (Fig. 8) and oleoyl LPE (Appendix IV) display similar fragmentation pattern to the corresponding PE. Once again, the M+H ion is the base peak. Protonated phosphoryl ethanolamine which was not seen in PEs was detected in both compounds. In

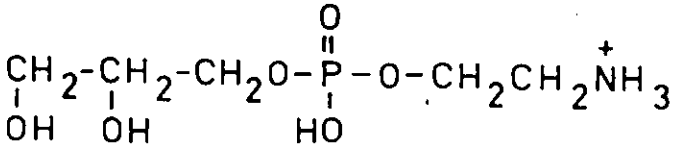
addition to those ions observed in corresponding PE, two more fragments may be useful for further characterization of this class of compounds, namely m/e 200 and m/e 216.



or



m/e 200 $[M-RCOO+2H]^+$



m/e 216

$[M-RCO+2H]^+$

Ions corresponding to protonated ethanolamine (m/e 62) as well as $[M+142]^+$ (m/e 621) and $[M+200]^+$ (m/e 680) were also seen in compound XXXII.

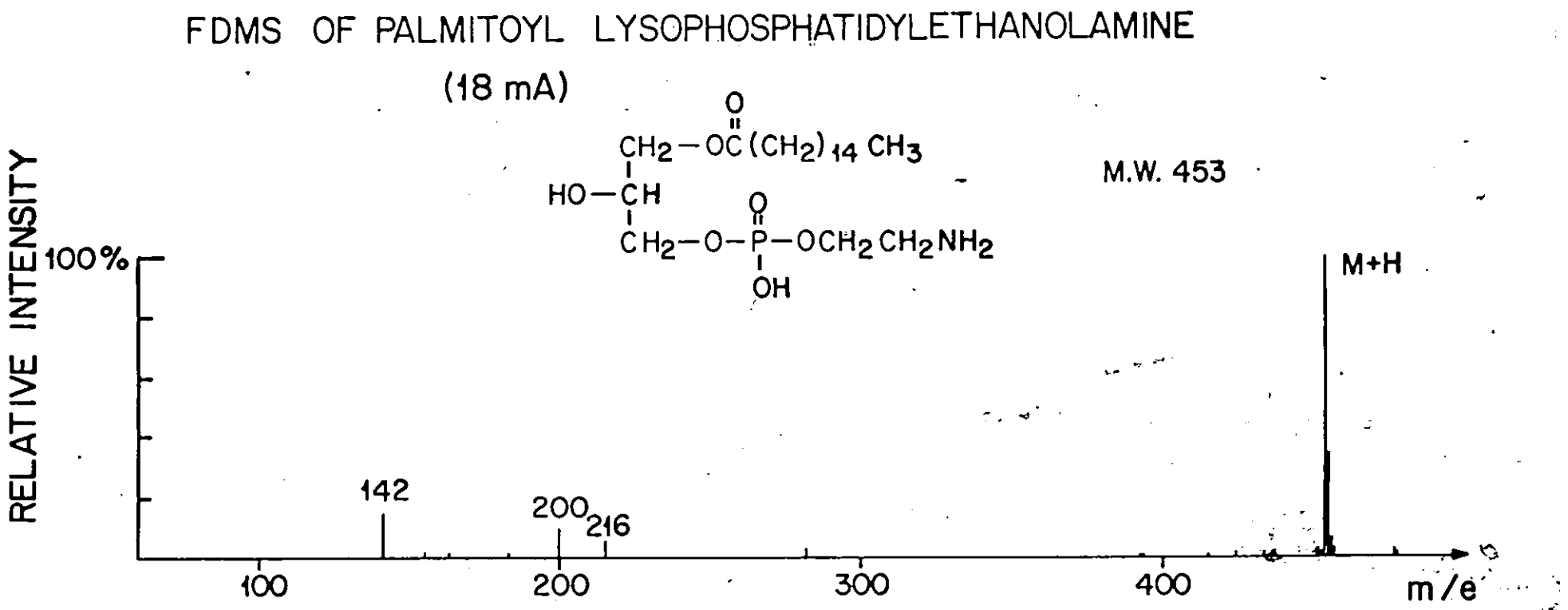


Fig. 12. Field Desorption Mass Spectrum of N-palmitoyl Dihydrosphingosine (XLVI).

E. Phosphatidyl N,N-dimethylethanolamine (PDME):

Dipalmitoyl phosphatidyl N,N-dimethylethanolamine field desorbed smoothly from the anode exhibiting the M+H ion (m/e 720) as the base peak (Table 10).

Table 10

Field Desorption Mass Spectrum of Dipalmitoyl Phosphatidyl N,N-dimethylethanolamine (XXXIII)[a]

XXXIII	R'=R"=$(CH_2)_{14}CH_3$	e.h.c.=18.5 mA	
m/e 170 (5%),	464(2,M-RCOO),	466(8,M-RCOO+2H),	
718(3,M-H),	720 (100,M+H),	721(46),	791(7,M+72)

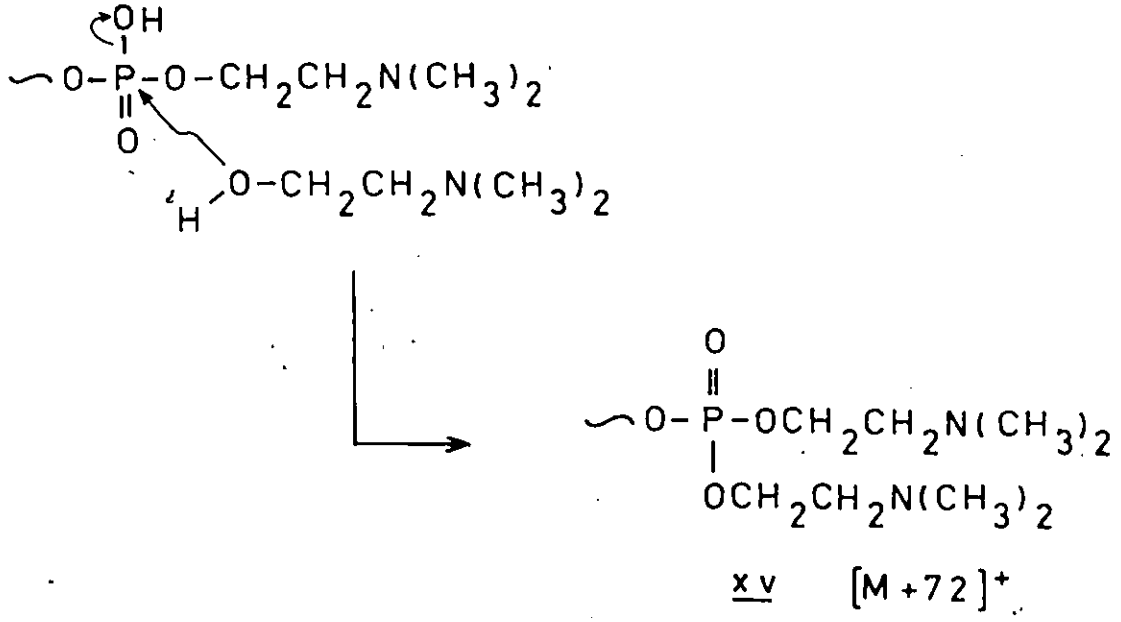
[a]Ions with rel. int. > 5% are listed.

[b]Each entry is of the form (m/e, rel. int.), and in some cases followed by the assignment

In apparent similarity to PC and PE, fragmentation of the molecular ion by simple bond cleavage yields protonated phosphoryl N,N-dimethyethanolamine (m/e 170), $[M-RCOO]^+$ (m/e 464) and $[M-RCOO+2H]^+$ (m/e 466). Significantly enough, neither $[M+15]^+$ nor $[M-15+2H]^+$ was detected. The criteria of a field-induced intermolecular methyl transfer in phospholipids are then established. (1) The methyl acceptor should exist in an anionic form. If it is protected by even a proton, the feasibility of the transfer process will greatly reduce. (2) The methyl donor will be better

carrying a positive charge. Among all the phospholipids that we have investigated, only choline containing compounds fulfill all these requirements.

One associated ion appeared at m/e 791 representing $(M+CH_2{=}\overset{+}{C}HNH(CH_3)_2)$ or structure xv.



xv $[M+72]^+$

Scheme 13. Rationalization of the Formation of $[M+72]^+$ (m/e 791) in f.d. Mass Spectra of Dipalmitoyl Phosphatidyl N,N-Dimethylethanolamine.

F. Phosphatidic Acids (PA) and Lysophosphatidic Acids (LPA):

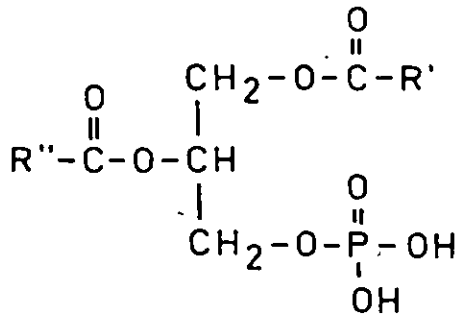


Chart 4

Cpd.	Name	Fatty Acid(s)	M.W.
XXXIV	Dicaproyl PA	R'=R"=$(CH_2)_8CH_3$	480.3
XXXV	Dipalmitoyl PA	R'=R"=$(CH_2)_{14}CH_3$	648.5
XXXVI	Caproyl LPA	R'=$(CH_2)_8CH_3$,R"=H or vice versa	326.1
XXXVII	Palmitoyl LPA	R'=$(CH_2)_{14}CH_3$,R"=H or vice versa	410.2
XXXVIII	Oleoyl LPA	R'=$(CH_2)_7CH=(CH_2)_7CH_3$, R"=H or vice versa	436.2

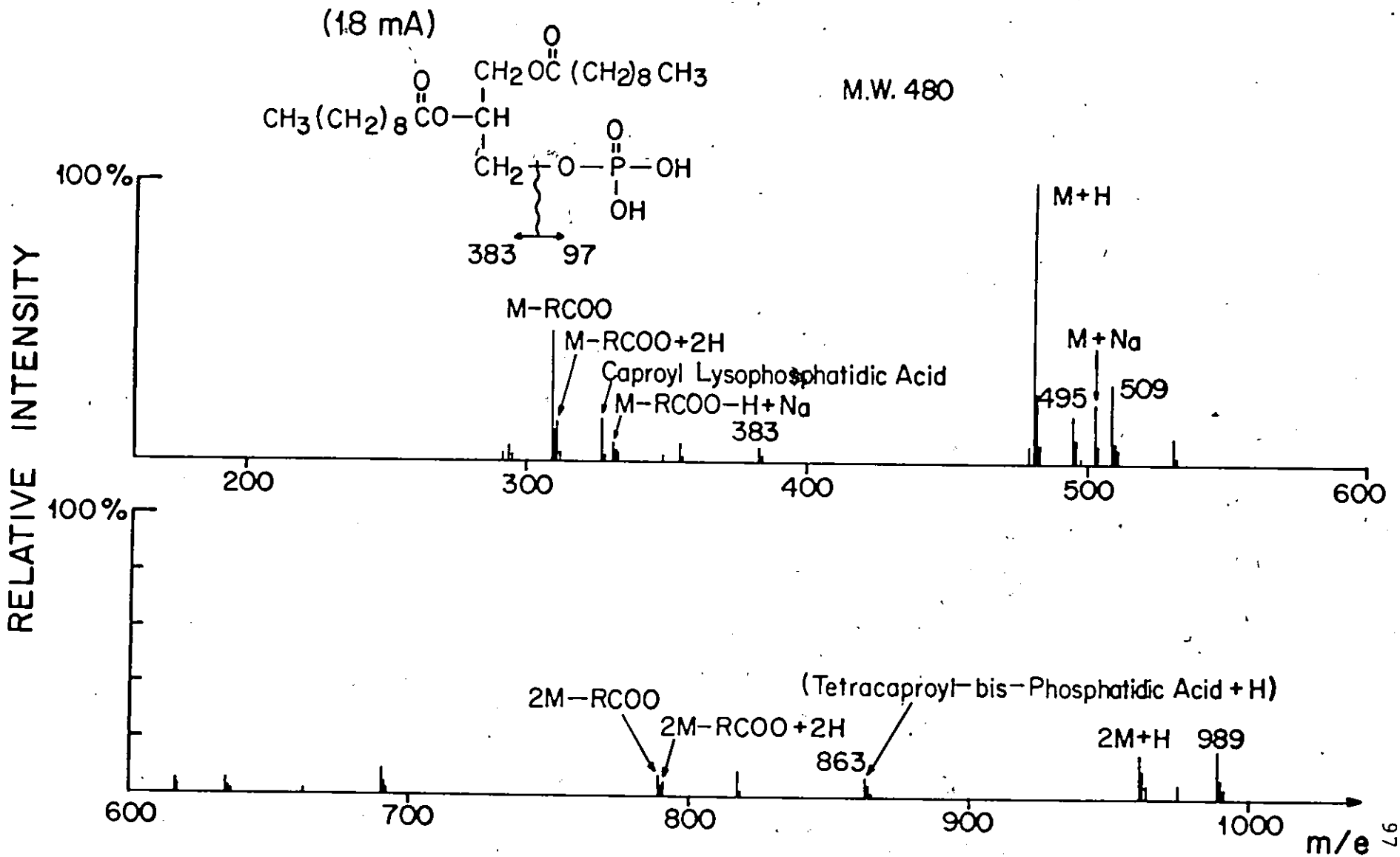
The analyses of phosphatidic acids (dicaproyl PA (XXXIV), dipalmitoyl PA (XXXV)) and lysophosphatidic acids (caproyl LPA (XXXVI), palmitoyl LPA (XXXVII), oleoyl LPA (XXXVIII)) indicated that each member of one class was contaminated with the corresponding compound from the other. Attempts had been made to purify them by acid treated florisil column chromatography[42] but decomposition of the sample was shown to occur during the chromatographic procedures. As a result, samples of PAs and LPAs should be regarded as a mixture of these two types of compounds. Nevertheless, in view of the fact that the BAT for LPAs (10-12 mA) is lower than that for PAs (14-18 mA), a "fractional desorption" technique can be used, in principle, to produce a f.d. mass spectrum of each component.

As an illustrative example of the f.d. mass spectrum of PAs, dicaproyl PA (XXXIV) yields a M+H ion (m/e 481) as the base peak at 18 mA (Fig. 9). The decay of $M^{+\cdot}$ or $2M^{+}$ into stabilized even-electron building blocks by elimination of small fragments in many organic compounds has also been observed. The resulting ions: $[M-RCOO]^{+}$ (m/e 309); $[M-RCOO+2H]^{+}$ (m/e 311); $[M-RCOO-H+Na]^{+}$ (m/e 331); $[M-H_2PO_4]^{+}$ (m/e 383); $[2M-RCOO+H]^{+}$ (m/e 780) and $[2M-RCOO+3H]^{+}$ (m/e 782) yield relevant structural information about the fatty acid composition and the polar group. The question whether the ion at m/e 327, corresponding to protonated caproyl LPA, derived from the compound itself by a loss of an acyl group or directly from the impurities is still unresolved. The correct assignment of the molecular weight is assisted by the occurence of the $[2M+H]^{+}$ ion.

Associated ion clusters in the upper mass range are assigned as follows: m/e 495 $[M+15]^{+}$, m/e 503 $[M+Na]^{+}$, m/e 509 $[M+29]^{+}$, m/e 617 $[M+155-H_2O]$, m/e 635 $[M+155]^{+}$, m/e 975 $[2M+15]^{+}$, m/e 990 $[2M+29]^{+}$. The ionic species with mass number 15 a.m.u. and 29 a.m.u. associated with the molecular ion or the 2M ion are tentatively assigned as methyl and ethyl group respectively, however, their origins are unknown. These two species were found in the f.d. mass spectrum of caproyl LPA (Appendix V) as well.

The structure of charged species m/e 155 and m/e 137 associated with the molecular ion may arise from cyclic

FIG. 9 FDMS OF DICAPROYL PHOSPHATIDIC ACID



glycerol phosphate and its dehydration product.

An ion at m/e 864 is attributed to the protonated tetracaproyl bis-phosphatidic acid.

The f.d. mass spectrum of dipalmitoyl PA (XXXV) was complicated by the presence of palmitoyl LPA (XXXVII) and dipalmitoyl PC (VI) (cf. Appendix V). At 14 mA the hydrated molecular ion is the most intense ion (118% where [M+H] = 100%). In addition, protonated and hydrated orthophosphoric acid (m/e 99 and 116 respectively) were also seen.

By comparison of the f.d. mass spectra of caproyl LPA (XXXVI), palmitoyl LPA (XXXVII) and oleoyl LPA (XXXVIII), several characteristic processes of these compounds are derived:

(1) Protonation of orthophosphoric acid (m/e 99) which is common for compounds containing a monosubstituted phosphate group as was observed for carboxyphosphamide[134], pyridoxamine 5'-phosphate hydrochloride[135] and nucleotides.[136]

(2) Hydration of H_3PO_4 (m/e 116).

(3) Formation of glycerophosphoric acid after hydrogen rearrangement (m/e 173 in XXXVI or m/e 172 in XXXVII and XXXVIII).

(4) Hydration of the acyloxy ion $[RCOO+H_2O]^+$.

(5) Loss of H_2O from $[M+H]^+$ and $[2M+H]^+$.

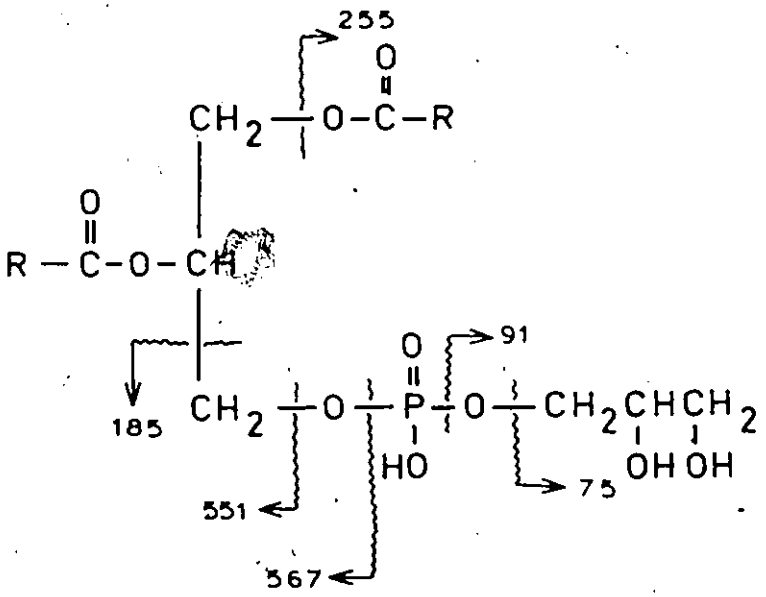
(6) Hydration of $[M+H]^+$ and $[2M+H]^+$ ($[M+H_2O]^+$ is the base peak in the spectrum of XXXVIII).

(7) Formation of a proton-bound complex with the molecular ion and phosphoric acid, $[M+H+H_3PO_4]^+$, as was observed in pyridoxamine phosphate.[135]

(8) Association of the molecular ion with charged species m/e 116 ($[H_3PO_4 \cdot H_3O]^+$), m/e 155 and m/e 173 (protonated glycerophosphoric acid).

In some individual cases, an intense ion of $[M+RCOO+H+H_2O]^+$ and protonated palmitoyl lysopyrophosphatidic acid (m/e 491) were detected in compound XXXVI and XXXVII respectively).

G. Phosphatidyl Glycerol (PG):



$R = (CH_2)_{14}CH_3$

At 14 mA, dipalmitoyl phosphatidyl glycerol (XXXIX) displayed a hydrated molecular ion ($M+H_2O$) as base peak in its f.d. mass spectra whereas the relative intensity of $[M]^+$

and $[M+H]^+$ were only ca. 6% (Table 11). However, at higher e.h.c. the proportion of $[M+H]^+$ significantly increased (e.g. at 15 mA, $[M+H]^+$ intensity increased to 35% as compared to 2% at 13 mA). This strong hydrate formation property can be readily rationalized by the presence of a diol in the free glycerol moiety. The latter has been believed to be highly susceptible to hydrogen bonding with water molecules. This argument is also supported by the presence of other hydrated ions as were observed in PAs and LPAs. Hydrogen bonding will be partially overcome at higher anode temperature by supplying more thermal energy, and consequently yielding higher $[M+H]^+$ intensity.

Table 11

Field Desorption Mass Spectrum of
Dipalmitoyl Phosphatidyl Glycerol[a]

XXXIX Dipalmitoyl PG, R=$(CH_2)_{14}CH_3$ e.h.c. = 14 mA
m/e 86 (5%)[b], 88 (9), 105 (8), 107 (3), 442 (5), 486 (3, M-RCOO+H_2O+H), 502 (4), 512 (4), 568 (7, dipalmitoyl glycerol), 569 (4), 586 (9, dipalmitoyl glycerol + H_2O), 587 (3), 591 (3), 722 (6, $[M]^{+\cdot}$), 723 (6,M+H), 740 (100, M+H_2O), 742 (8), 764 (4), 810 (6, M+88) 811 (4).

[a] All ions with relative intensity greater than 3% are reported.

[b] Each entry is in the form of m/e (rel. int.) and in some cases followed by the assignment.

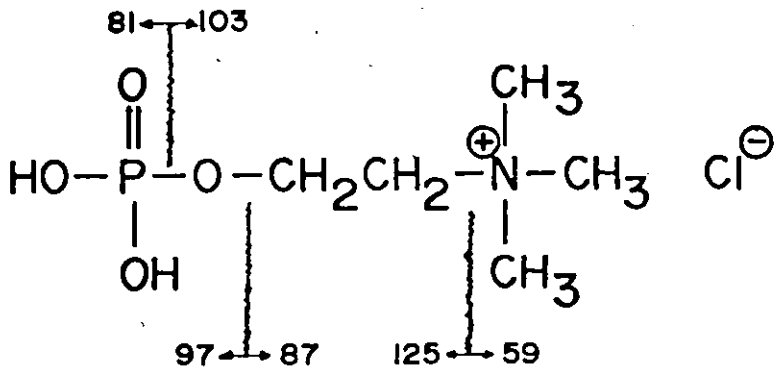
H. Phosphoryl Choline Chloride and Phosphoryl Ethanolamine

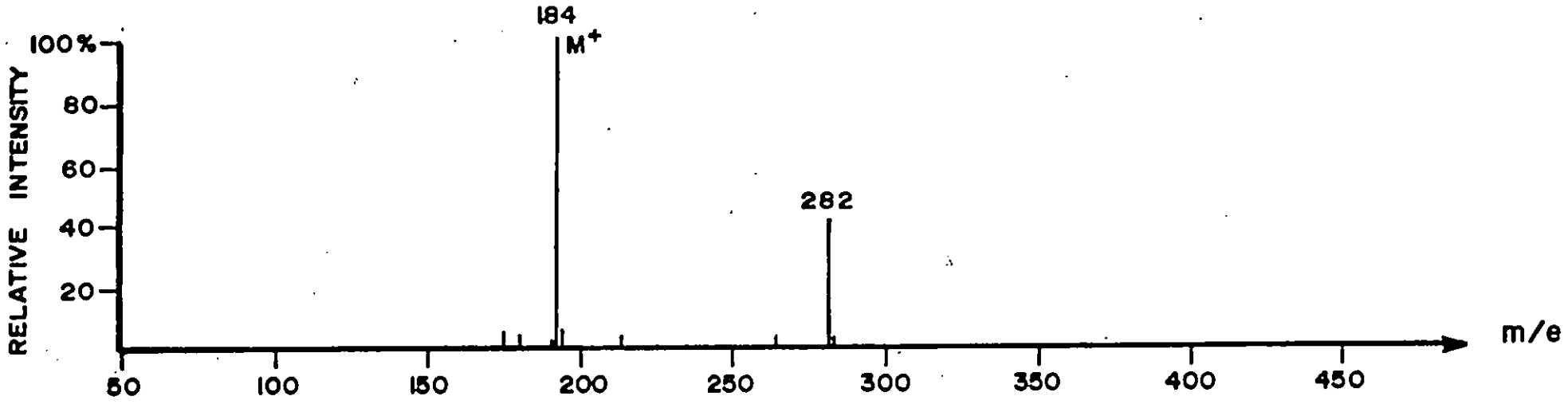
Phosphoryl choline chloride (XL) prepared from its calcium salt was submitted for f.d.m.s. analysis. As shown in Fig. 10, an intense molecular ion $[M]^{+\cdot}$ was found. The occurence of $[M+CH_3-H]$ and $[M-CH_3+H]$ provides further evidence for the intermolecular methyl transfer as described previously in PC. The ion corresponding to M+99 could arise from the hydrated pyrophosphoryl choline or the association complexes of the molecular ion and a phosphoric acid ($M+H_3PO_4$).

The f.d. mass spectrum of phosphoryl ethanolamine (XLI) (phE) (Fig. 11, an average of 5 scans with e.h.c. between 10-11 mA) is a good example to illustrate the field-induced phosphorylation which appears to take place in many organo-monophosphates. The proposed mechanism is shown in Scheme 14, where condensation of two phE molecules (one is protonated) leads to the formation of an aminoethyl pyrophosphoric acid (m/e 222) which in turn eliminates the aminoethyl group and generates a pyrophorphoric acid (m/e 179) and its hydrated species (m/e 197). Further condensation of aminoethyl pyrophosphate with another molecule of phE gives rise to aminoethyl triphosphate ion at m/e 302. This kind of phosphorylation process happens even at low anode temperature (10-11 mA) implying that it is indeed a field-induced solid state reaction. High resolution data of D-glucose-6-phosphate and deoxygluoro-D-glucose-phosphates[137] has also shown the existence of those phosphorylation products.

Fig. 10. Field Desorption Mass Spectrum of Phosphoryl Choline Chloride (XL).

PHOSPHORYL CHOLINE CHLORIDE (10 mA) (#2)





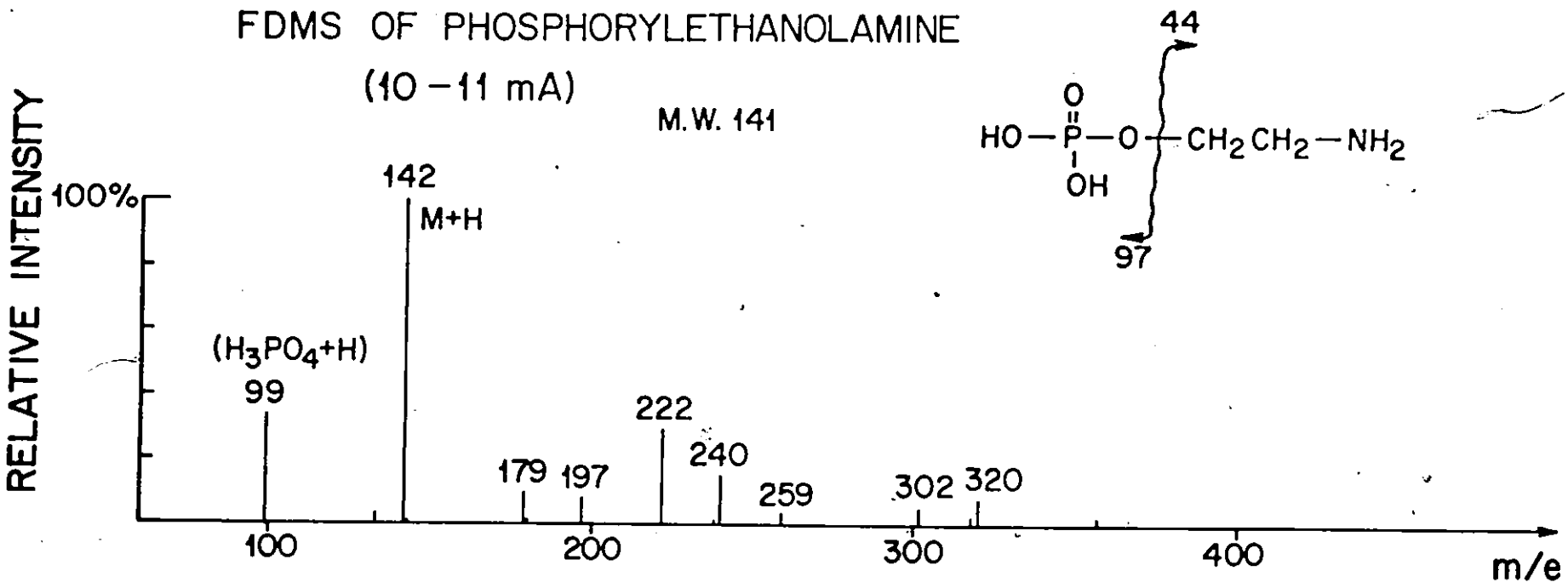
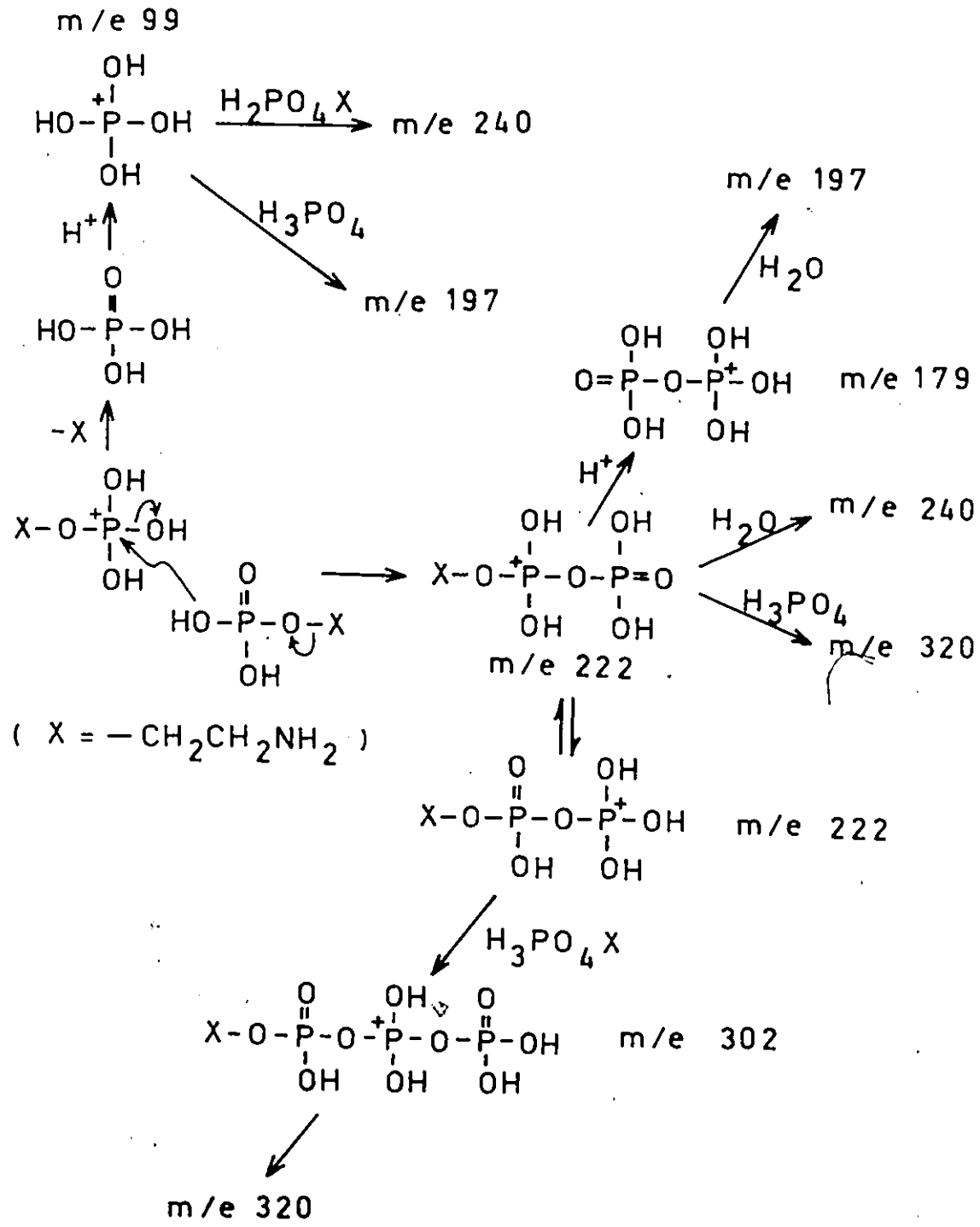


Fig. 11. Field Desorption Mass Spectrum of Phosphoryl Ethanolamine (XLI).



Scheme 14. Proposed Mechanism for Field-Induced Phosphorylation in f.d.m.s. of Phosphoryl Ethanolamine.

It is still difficult to define whether the structure of the ions at m/e 197, 240 and 320 are hydrated species or proton-bound complexes of H_3PO_4 with phosphoric acid, phosphory ethanolamine and aminoethyl pyrophosphate respectively. Perhaps both forms make some contribution.

I. Glycerophosphoryl Amino Alcohols:

Table 12 presents the f.d. mass spectra of glycerophosphoryl choline (XLII), glycerophosphoryl ethanolamine (XLIII) and glycerophosphoryl serine (XLIV).

In spite of the absence of the molecular ion in the spectrum of (XLII) the compound still may be identified by $[M+Na]^+$ (m/e 280) as the base peak in the high mass region as well as the $[2M+Na+H]^+$ (m/e 538, 16%) and $[M+K]^+$ (m/e 296, 3%). The doubly charged ion $[M+2Na]^{2+}$ may account for the peak at m/e 151.5. The most intense signal at m/e 104 (170%, choline) may result from cleavage between the choline oxygen and the phosphorus or directly from impurities.

At 18 mA, $[M+H]^+$ (m/e 216) in the spectrum of XLIII has a relative intensity of only 68%. The structure of the base peak at m/e 242, 27 a.m.u. higher than the molecular ion is not well understood.

Other ions such as $[M+Na]^+$ (22%), $[phE+H]^+$ (m/e 142, 6%), [phE-H+Na] (m/e 164,11%) are also seen.

Table 12

Field Desorption Mass Spectra of Some Glycerophosphoryl Amino Alcohols[a]

XLLII Glycerophosphoryl Choline M.W. 257.2 e.h.c.=17 mA
m/e 88[b] (9%, $[CH_3CH_2\overset{+}{N}(CH_3)_3]$), 103 (170), 105 (8), 151.5 (7, $[M+2Na]^{++}$), 186 (2), 254(10), 280 (100, M+Na), 281 (8), 296(3, M+K), 361 (9, M+104), 457 (5), 488 (9), 538 (16, $[2M+Na^+ +H]$).

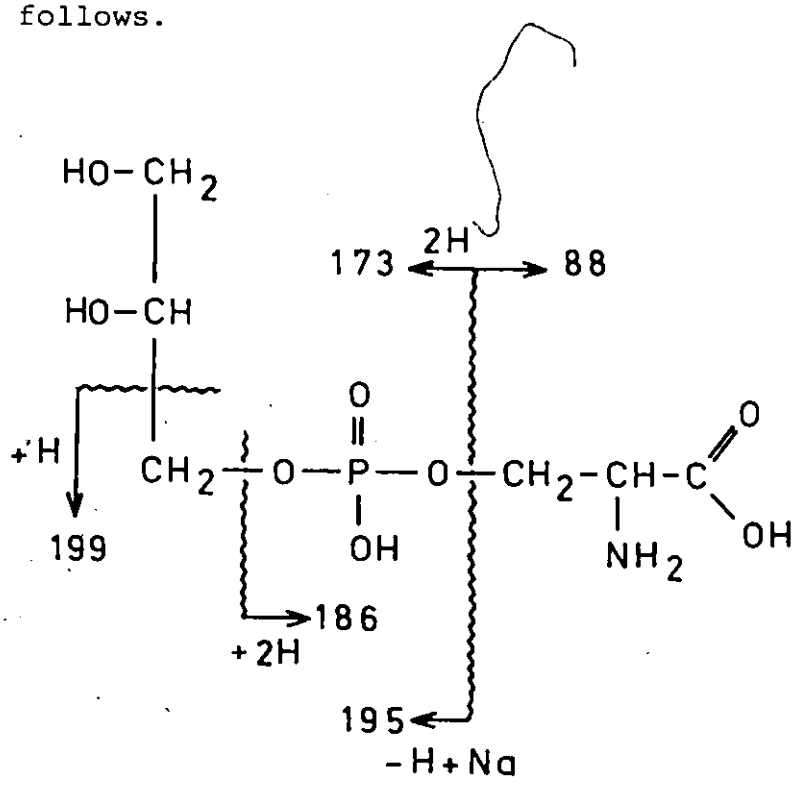
XLIII Glycerophosphoryl Ethanolamine M.W. 215.2
e.h.c.=18 mA
m/e 142 (6%, $[H_2PO_4CH_2CH_2\overset{+}{N}H_3]$), 164 (11, m/e 142-H+Na), 216 (68, M+H), 217 (4), 238 (22, M+Na), 242 (100), 243 (13).

XLIV Glycerophosphoryl Serine M.W. 259.2 e.h.c.=16 mA
m/e 23 (33%, Na^+), 39 (6, $^{39}K^+$), 99 (36, H_3PO_4+H), 121 (22, NaH_2PO_4+H), 171 (9), 173 (43, glycerophosphoric acid +H), 177 (6), 193 (13), 195 (100, glycerophosphoric acid +Na+H), 197 (9), 219 (13), 260 (3,M+H), 261 (7,M+2H), 267 (11), 271 (9), 284,(11), 293 (7).

[a]All ions with relative intensities greater than 5% and others of particular interest are reported.

[b]Nominal masses after mass defect correction.

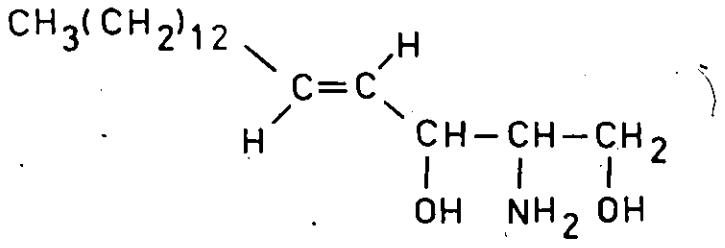
From our experience, the f.d. mass spectra of serine containing compounds always present some difficulties for mass spectral interpretation. The $[M+H]^+$ intensity is normally low or even absent and the fragment rich "e.i.-like" spectra make the identification more difficult. A suggested fragmentation scheme for glycerophosphoryl serine (XLIV) is presented as follows.



Scheme 15. Fragmentation Scheme of Glycerophosphorly Serine in f.d.ms.

J. Sphingolipids:

Spingosine (XLV), a long-chain monounsaturated amino glycol and its saturated analog, dihydrosphingosine (sphinganine), are the building blocks of all sphingolipids.



As shown in Table 13, sphingosine desorbed smoothly from the f.d. anode at low e.h.c. (9 mA) giving a simple spectrum with $[M+H]^+$ as the base peak. This is in marked contrast to the e.i. mass spectrum of its 1,3-di-O-TMS-N-acetyl derivative[138] in which the molecular ion is absent. In addition, diagnostic ions $[2M+^{39}K,^{41}K]$ and $[M+2H-NH_3]$ were also observed.

Table 13

Field Desorption Mass Spectra of Some Sphingolipids

XLV Sphingosine M.W. 299.3 e.h.c.=9 mA

m/e 286 (3,$M+2H-NH_3$), 298 (4,M-H), 300 (100, M+H), 301 (22), 302 (4), 329 (3,$M+CH_2OH-H$, phytosphingosine), 638 (17,$2M+^{39}K^+$), 639 (7), 640 (8,$2M+^{41}K$)

XLVI N-Palmitoyl dihydrosphingosine, M.W. 539.5

e.h.c.=18 mA.

m/e 239 (28, RCO+H), 241 (7), 298 (4), 509 (8,$M-CH_2OH+H$), 510 (6), 521 (3,$M-H_2O$), 538 (16,M-H), 539 (8), 540 (100, M+H), 541 (43), 542 (8), 543 (3) cont'd

Table 13 cont'd

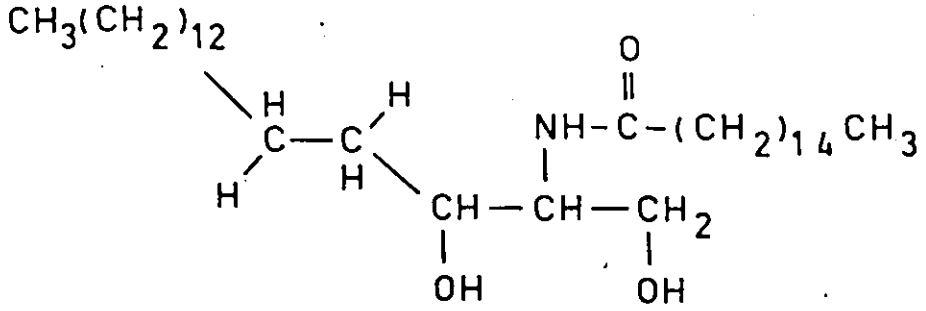
Field Desorption Mass Spectra of Some Sphingolipids

XLVII	Psychosine	M.W. 461.2	e.h.c.=11 mA

m/e 434 (3%), 462 (100, M+H), 463 (32), 464 (13), 465 (3), 484 (8,M+Na), 500 (9,M+K), 502 (4), 923 (7,2M+H), 924 (4).

[a]All ions with relative intensities greater than 3% are reported.

Ceramides, which are the derivatives of sphingosine and dihydrosphingosine, possess a long-chain fatty acid in amide linkage with the nitrogen of the amino glycerol.



XLVI

The f.d. mass spectrum (Fig. 12) of N-palmitoyl dihydrosphingosine (XLVI) at 18 mA parallels that of XXXXV in that the $[M+H]^+$ ion is the most intense ion. Charge retention on the C_3 atom from the cleavage between C_2-C_3

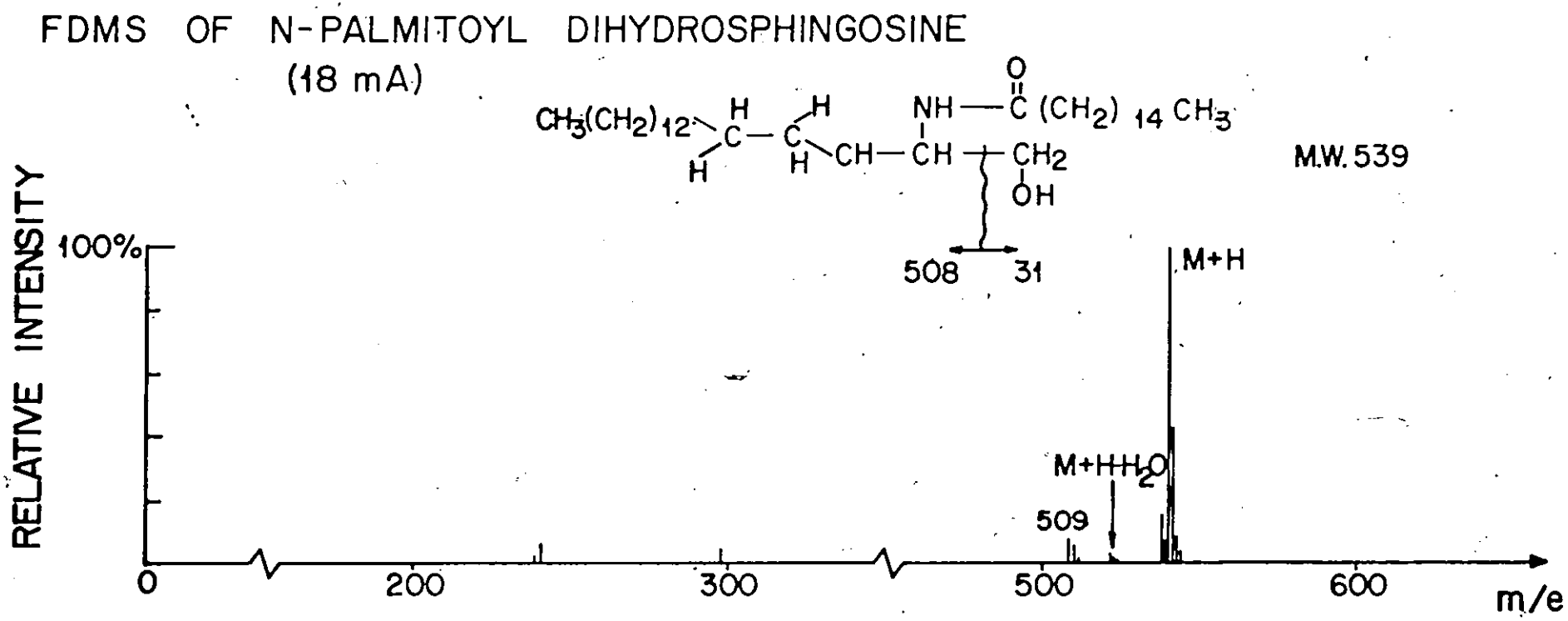
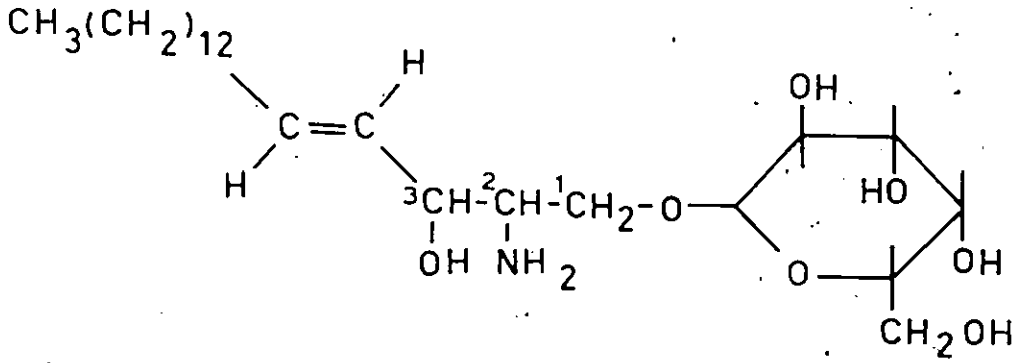


Fig. 12. Field Desorption Mass Spectrum of N-palmitoyl dihydrosphingosine (XLVI).

bond leads to m/e 241. Similar cleavage has been observed in 1,3-bis-O-TMS-N-stearoyl sphingosine and 1,3-O-TMS-N-oleoyl dihydrosphingosine.[62,139,140] The spectrum also shows an ion at m/e 509 resulting from the bond fission between C_1 and C_2 bond followed by hydrogen rearrangement. Loss of water from M+H has about 3% relative intensity. It should also be noted that the ion at m/e 537 may be attributed to either $[M-H]^+$ or protonated molecular ion of N-palmitoyl sphingosine, the later being a likely impurity.

Psychosine (XLVII) is another derivative of sphingosine in which the hydroxyl group at position 1 is attached to a molecule of galactose.



XLVII

The molecular weight of XLVII can be unambiguously identified by $[M+H]^+$ (m/e 462) ion as well as $[M+Na]^+$ (m/e 484), $[M+K]^+$ (m/e 500) and $[2M+H]^+$ (m/e 923) in its f.d. mass spectrum.

CHAPTER V

CONCLUSIONS

This work demonstrates that f.d.m.s. is a very promising technique in the analysis of nonvolatile and thermally labile phospholipids. In general, the f.d. mass spectra of these compounds have the following characteristic features.

(1) Protonation of sample molecules by solvent or by the acidic moiety of another molecule in the adsorbed surface layer results in the $[M+H]^+$ even electron species which, in most cases, is the base peak of the spectrum.

(2) In respect of the high intensities of even-electron $[M+H]^+$ or cationized molecular ion (eg. $[M+Na]^+$) obtained, f.d.m.s. shares the advantage of c.i.m.s. which is in contrast to e.i.m.s. where primary ionization of molecules in the gaseous phase results in odd-electron molecular ions $[M]^+$ with relatively low activation energy for fragmentation. This factor, together with others such as minimal energy transfer in the ion production process and short residence time of ion in the f.d. ion source, both greatly simplify the mass spectrum for most compounds and make their interpretation easier.

(3) At higher temperatures than the BAT, most structurally relevant information can be obtained by the outstanding and commonly observed field-induced direct bond ruptures in the sample molecules. Additionally, the frequent appearance of complementary, charged particles simplifies the interpretation of the f.d. mass spectrum considerably.

(4) The decay of the molecular ion into stabilized even-electron ions by elimination of small, neutral particles has also been observed.

(5) For a thick layer of adsorbed molecules on the emitter surface in which the polar group of the molecules interact extensively with each other, field-/ thermally-induced multistep rearrangement reactions and associated ion cluster formation between the molecular ion and charged fragment are also significant.

(6) Compounds containing a free phosphate group usually undergo field-induced phosphorylation and yield a characteristic ion $[H_3PO_4+H]^+$ at m/e 99 at high e.h.c.

(7) For molecules or ions with strong hydrogen bonding properties, it is possible to observe their hydrated species.

(8) Doubly charged ions and (2M+H) or (2M+Na) ions are also detected.

(9) Intermolecular methyl transfer has been confirmed in choline containing compounds.

(10) Emitter temperature has crucial influence on these processes and therefore on field desorption mass spectra.

Comparison of low resolution f.d. mass spectra of this selection of phospholipids allows the construction of straightforward schemes for formation of most of the major ions. Reference has been made to previous e.i. work and high resolution f.d. spectra where possible. While it is unlikely that the schemes presented here are correct in every detail, present experience with f.d.m.s. suggests that it is much less subject to the profound and often unpredictable rearrangements and reorganizations of molecules common to e.i.m.s. The mechanisms and structures presented are thus considered to be reasonable hypotheses and a necessary platform from which more detailed mechanistic work may be launched. Research is now underway to apply this powerful analytical technique in determination of phospholipids in amniotic fluid and other medical problems.

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PART II

CHARACTERIZATION OF MODIFIED NUCLEOSIDES AND NUCLEOTIDES BY FIELD DESORPTION MASS SPECTROMETRY

CHAPTER I

INTRODUCTION

The major aim of this research is the utilization of f.d.m.s. for identification of modified nucleic acid bases, nucleosides and nucleotides from nucleic acid hydrolysates. This is particularly important in studies of the interaction of chemical carcinogens or their activated metabolites with nucleic acids, both DNA and RNA. In this chapter, a brief review on the modification of nucleic acid components by chemical carcinogens and mutagens and a review of mass spectrometry of various nucleosides and nucleotides will be presented.

It is generally agreed that most human cancers may be caused by chemicals.[1] Considerable evidence has shown that these chemical carcinogens (either as such or after suitable metabolic activation) are covalently bound to DNA, and other cellular marcomolecules[2] (RNA and proteins). Some of these agents are also mutagenic.[3] Among the potent and well-understood chemical carcinogens are alkylating agents, such as alkyl sulfates, alkyl sufonates, alkyl halides, dialkyl nitrosamines, alkyl nitrosoureas, acyl nitrosamides, mustards, diazo compounds, lactones, and epoxides.[4] The chemical basis of nucleic acid modification in relation to carcinogenesis and mutagenesis has been reviewed recently by several workers.[1-6] It is generally believed that 1) all chemical carcinogens

other than active alkylating agents must be converted metabolically into their chemically reactive forms and 2) these activated metabolites are electrophiles (electrophilic agents) which in turn react with nucleophilic groups in cellular macromolecules to initiate carcinogenesis. As electrophiles or potential electrophiles the carcinogens have common nucleophilic targets which include all four bases and in some instances the phosphodiester backbone of the nucleic acids. The N-7 of guanine appears to be the most reactive site followed by the N-3 and N-7 position of adenine. To date, interaction at the following sites have been reported: N-7, O-6, N-3, 2-NH_2 and C-8 of guanine; N-1, N-3, N-7 and 6-NH_2 of adenine; N-1, N-3 and C-5 of cytosine; O-4 and C-6 of thymine and N-3 of uracil (Fig. 1.A, B, C). Much of this work has been the subject of several excellent reviews.[2,4,7-9] The quantitative and qualitative nature of the modified products varies with the agent, the type of nucleic acids and the organism used.

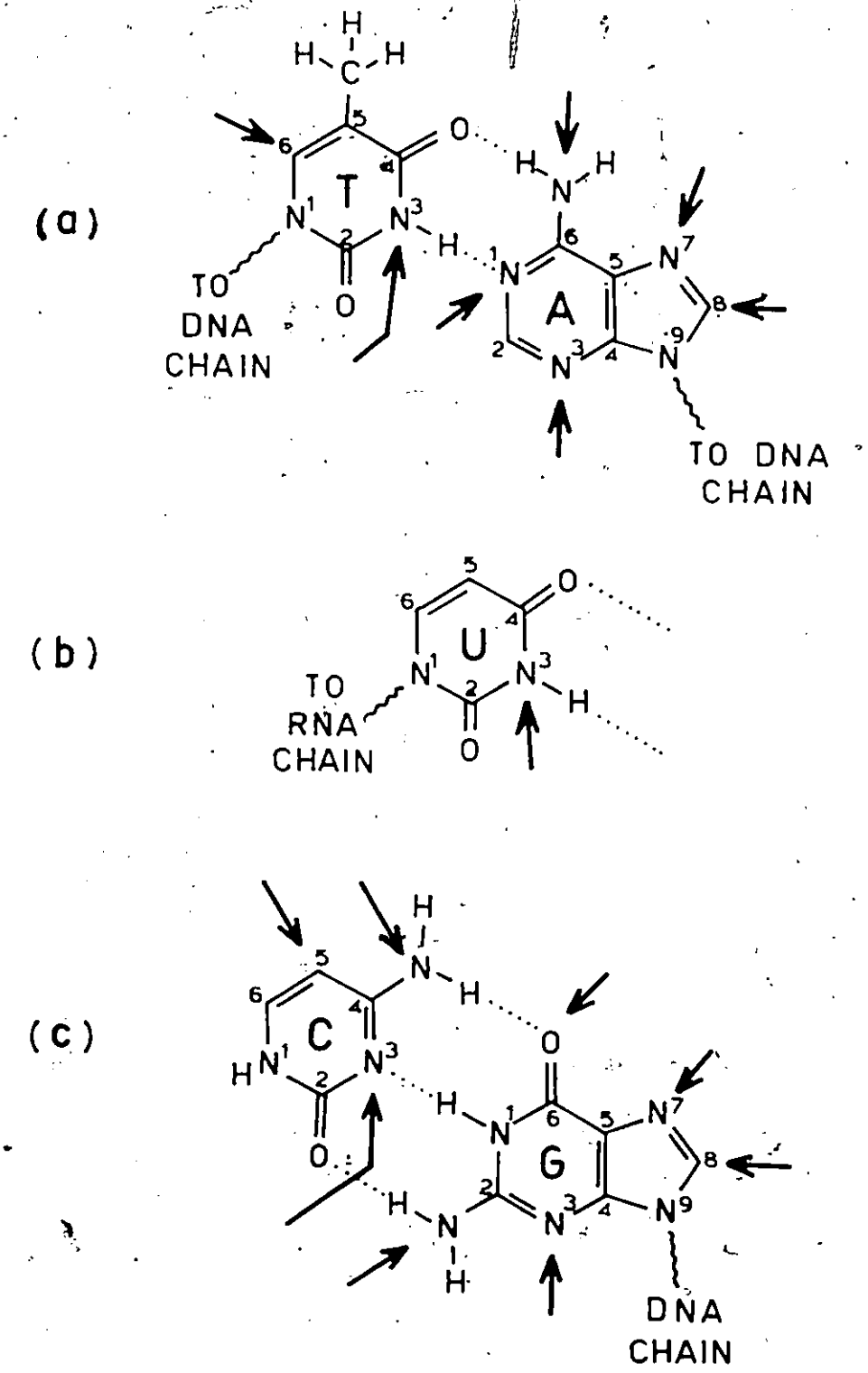


Fig. 1. Possible Reaction Sites for Alkylation of Nucleic Acid Components.

Although great advances have been made in this area, the fact stands out that the nature of the reactive form and the mode of binding to the nucleic acids are still not clearly established. It should be emphasized that a number of compounds may be covalently bound to DNA *in vivo* under conditions which do not lead to the induction of cancer, so that other factors must be important. More studies on the qualitative aspects of the interaction of carcinogens with nucleic acids (especially DNA) need to be carried out to determine whether or not substitution at particular sites of bases in DNA is critical in carcinogenesis. For this reason, a sensitive and specific analytical technique is necessary for identification of chemical changes induced by carcinogens, particularly for some of the minor modified bases which may be biologically significant.

The classical approach to the identification of nucleic acid bases and their derivatives after chemical or enzymatic degradation was based on the well-established radioisotope labelling,[10] spectrophotometric (including widely used u.v. absorption spectrophotometry,[4,11] fluorimetry, i.r. spectrophotometry), and chromatographic (including t.l.c., paper chromatography, liquid chromatography and recently g.l.c.) techniques. However, these techniques all suffer from a lack of sensitivity and/or specificity. Verification of the structure of a minor nucleic acid constituent is only possible by comparison with an authentic compound.

Mass spectrometry, which has been widely utilized in the structure elucidation of many classes of organic compounds, appears to satisfy both the requirements of high specificity and sensitivity. That nucleosides are amenable to electron impact mass spectrometry (e.i.m.s.) was shown by Biemann and McCloskey[12] in 1962. These compounds exhibit a rather straightforward fragmentation pattern (Fig. 2) that could be used to identify the base and also provide considerable information about the sugar moiety.[11,13-15]

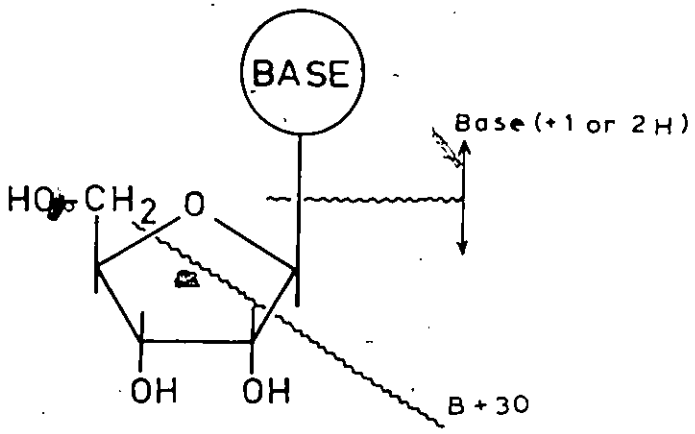


Fig. 2. Fragmentation Scheme of Nucleosides in e.i.m.s.

The major limitation in the application of e.i.m.s. to nucleic acid chemistry is the requirement that prior to electron bombardment, the sample should be vaporized without thermal decomposition. This is particularly significant for nucleotides because of the presence of a phosphate ester bond. The effect of structural modification upon sample volatility and thermal stability depends on whether

opportunities for hydrogen-bonding interaction have been increased or decreased. For instance, deoxyribonucleosides are more volatile than the corresponding ribonucleosides, and O-methylation and N-methylation usually increase volatility.[14] Various types of derivatives[13,14] (TMS,[16] N,O-permethyl derivatives,[17] acetates, trifluoroacetates, acetonates, phenylboronic acid esters[18,19]) have been used to enhance the volatility of nucleosides and nucleotides by blocking the sugar hydroxyl and phosphate groups, and sites of labile hydrogen in the base. Some of these derivatives have sufficient volatility for g.l.c.-m.s. analysis. However, chemical derivatization has several inherent disadvantages. These include: 1) large amount of sample (in the order of mg) required for chemical derivatization, particularly limiting for the identification of minor modified components; 2) incompleteness of reaction leading to a multiplicity of products or less than quantitative yield; 3) ready hydrolysis of some derivatives and 4) substantial increase in molecular weight which occurs upon derivatization of polyfunctional compounds. For example, with a trimethylsilyl (TMS) group (72 mass units) added per functional group, the molecular weight of 5'-AMP increases from m/e 347 to m/e 707 (5'-AMP-$(TMS)_5$). Several recent review articles have been published[11,13-15] concerning e.i.m.s. of the five major bases, nucleosides, nucleotides and their analogues, both free and chemically derivatized.

Examination of nucleic acid hydrolysates for normal and modified components has been made through time of flight mass spectrometry.[20,21] Alternatively, the TMS and trifluoroacetate (TFA) nucleoside derivatives in unfractionated DNA hydrolysate can be identified by high resolution e.i.m.s.[21,22] Pyrolysis of DNA on the direct probe of an e.i. mass spectrometer has been shown[23,24] to result in ions derived from the four common bases.

Brown et al[25] first recorded the field ionization (f.i.) mass spectra of a number of nucleosides and compared them to their e.i. spectra. The f.i. spectra are extremely simple, with all except guanosine (highest mass peak corresponding to $[M-H_2O]^+$) showing intense molecular ion peaks and characteristic sugar and base (plus hydrogen) fragments as the only other significant ions. Identification of the purine and pyrimidine bases by f.i.m.s. in nucleic acid hydrolysates was also reported.[26]

Chemical ionization (c.i.) mass spectra of nucleosides[27-29] and their analogues[30] also display an enhanced M+H ion at the expense of many diagnostic fragments ions. This technique has been used to differentiate isomeric 7- and 9-β-D-ribofuranosyl purines by comparing the relative intensity of the $[BH_2]^+$.[31] Hydrolysates of oligonucleotides obtained from transfer RNA have also been analyzed after permethylation.[29]

In spite of the small energy transferred during the ionization process for both f.i.m.s. and c.i.m.s., they possess a major disadvantage of e.i.m.s.: the sample must be vaporized, frequently after chemical derivatization. Since field desorption mass spectrometry was introduced by Beckey,[32] this novel technique has widened the scope of biological application of mass spectrometry as demonstrated by promising applications to thermally labile and nonvolatile compounds (see recent review by Beckey and Schulten[33] and references cited therein). Three main advantages of this method, namely, small sample consumption, high molecular ion intensities and controlled fragmentation enable the f.d.m.s. to be a promising method for the identification of nucleic acid components. Schulten and Beckey[34] first demonstrated that this method can handle underivatized nucleosides and nucleotides. Adenosine, thymidine and 5'-monophosphates of adenosine, thymidine, deoxycytidine and deoxyguanosine all display prominent molecular ion and fragment ions corresponding to the base and the sugar moiety. Guanosine, which decomposes close to evaporation temperature, does not give a molecular ion in its f.i. spectrum but does give an abundant $M^{+\cdot}$ (20% of $[B+H]^+$ = 100%) in f.d.m.s. Recently, the f.d. mass spectrum of the sodium salt of cyclic adenosine monophosphate has been reported.[35] Pyrolysis-f.d. has been carried out on herring DNA resulting in the identification

of cytosine, methylcytosine, thymidine, adenine and guanine as well as some nucleosides, nucleotides and dinucleotides.[36]

In view of the great potential of f.d.m.s. in identification of nucleic acid components, especially in pre-fractionated nucleic acid hydrolysates, and in order to test the applicability and versatility of the analytical procedures, f.d.m.s. of various normal and methylated nucleic acid bases, nucleosides and nucleotides were investigated and reported herein. In application of this method to the cancer problem, comparison of the structure of the modified nucleic acid component with that of the original carcinogen should give some indication of the nature of metabolic activation and thus the structure of the ultimate carcinogen.

CHAPTER II

EXPERIMENTAL

A. Instrumental

Mass spectra were obtained in the same manner as described in the experimental section of Part I of this dissertation (p. 22). Solvents used for dipping are listed in TABLE 1.

Table 1

Solvents Used to Prepare Sample Solutions for Dipping

Compounds	Solvent
2-MeAde, 3-MeCyt 1-MeUra, A, C, T U, 1-MedA·HCl, 5-MeC 3-MeC, dC, 1-MeA dU, 3-MeU, N^3-MeT 5-MeU, dC·HCl, N^6-MedA N^6-MeA	H_2O
1-MeAde, 7-MeGua 6-MeUra, C, G N_2-MeG, dA, 7-MeG dG, 1-MedG, 1-MeG	DMSO (dimethyl sulfoxide)
All nucleotides	0.2% $(NH_4)_2CO_3$

B. Chemicals

6-Methyluracil, 7-methylguanine, 1-methyladenine, 2-methyladenine (hemi-sulfate), N^2-methylguanosine, 2'-deoxyguanosine, 5-methylcytidine, 7-methylguanosine, 2'-deoxyuridine, 5-hydroxymethyl-2'-deoxyuridine, 3-methyl-uridine, adenosine-5'-monophosphate, cytidine-5'-monophosphate were purchased from Sigma Chem. Co., St. Louis, Mo.

5-Methylcytosine·$\frac{1}{2}H_2O$, adenosine, cytidine, uridine, thymidine, guanosine, 3-methylcytidine, 2'-deoxycytidine hydrochloride salt, 2'-deoxycytidine, 2'-deoxyadenosine, 1-methyl-2'-deoxyadenosine hydrochloride salt, 5-methyl-uridine, 2'-deoxyguanosine, adenosine-3'(2')-monophosphoric acid (monohydrate) were purchased from Calibiochem. Inc., San Diego, Calif.

N^6-Methyladenosine, N^6-methyl-2'-deoxyadenosine and 1-methyl-2'-deoxyguanosine (hemihydrate) were purchased from Cyclo Chem., Los Angeles, Calif.

Dibenzo-18-crown-6 ether was purchased from Aldrich Chem. Co., Milwaukee, Wis.

AG 50W-X8 (20-50 mesh), AG 11A8 (50-100 mesh), AG 501-X8 (20-50 mesh) ion-exchange resins were purchased from Bio-Rad Laboratories, Richmond, Calif.

Amberlite IR-120H was purchased from BDH Chem. Co. Poole, England.

All solvents and other reagents are A.C.S. grade and were used without further purification.

C. Ion-Exchange Chromatography

(1) Preparation of Uridine Model Solution:

Uridine (10.0 mg) and NaCl (12.0 mg) were dissolved in 2.0 ml deionized water. The molar ratio of uridine to NaCl of the resulting solution is 1 to 5.

One ml of this solution was submitted for f.d.m.s. analysis to study the suppression effect of the sodium ion. Another ml was subjected to ion-exchange chromatography for desalting.

(2) Preparation of Column:

Approximately 3 gm of the resin (Bio-Rad AG 50W-X8 or AG 501-X8, or AG 11A8, or Amberlite IR-120H) slurried in deionized water was packed in a disposable column (0.7 x 15 cm) and was thoroughly washed with deionized water. Twenty ml 2% HCl was passed through the column in order to make sure all the ion-exchange resins were in hydrogen form. The column was subsequently washed with deionized water until a negative Cl^- test was shown by $AgNO_3$ solution. One ml of the model solution was then applied on the column and eluted with deionized water. One-ml fractions were collected and tested by silica gel t.l.c. (solvent MeOH:H_2O). Fractions that gave a single spot were combined and concentrated to ca. 0.25 ml, and was subsequently submitted to f.d.m.s. analysis.

It should be mentioned that all glassware used was siliconized.

D. Solvent Extraction of DNA Hydrolysate by Dibenzo-18-Crown-6-ether

The DNA hydrolysate sample was extracted twice with equal volumes of 1X 10^{-2}M dibenzo-18-crown-6 ether in $CHCl_3$. Phase separation was achieved by means of centrifugation. The aqueous layer was concentrated to ca. 1 ml under vacuum and submitted for f.d.m.s. analysis. All glassware used was siliconized.

CHAPTER III

RESULTS AND DISCUSSION

A. Methylated Purine and Pyrimidine Bases

Field desorption mass spectra of 6 methylated purine and pyrimidine bases, namely 1-methyladenine (1-MeAde); 2-methyladenine (2-MeAde), 7-methylguanine (7-MeGua); 3-methylcytidine (3-MeCyt); 1-methyluracil (1-MeUra), and 6-methyluracil (6-MeUra) are extremely simple. They all display either $M^{+\cdot}$ or $[M+H]^+$ (in the case of 3-MeCyt) as the base peak. The results shown in Table 2 indicate that none of the spectra contains any fragment ions.

Table 2

Field Desorption Mass Spectra of Methylated Purine and Pyrimidine Bases

Compound	1-MeAde	2-MeAde	7-MeGua	3-MeCyt	1-MeUra	6-MeUra
Mol. Wt.	149	149	165	125	126	126
Solvent	DMSO	H_2O	DMSO	H_2O	H_2O	DMSO
e.h.c.	17mA	16mA	20mA	15.5mA	8mA	15mA
M	100%[†]	100%	100%	71%	100%	100%
M+1	15%	13%	9%	100%	5%	7%
M+2				5%		
2M-1		6%				
2M+1				15%		

[†]Relative Intensities

The high intensity of the molecular ion reveals that an electron can be easily removed from the molecule and the resulting radical is stabilized by the highly aromatic nucleus. However, the prominent M+H ion in the f.d. mass spectrum of 3-methylcytosine suggests another pathway for the ionization of this compound. In view of the presence of abundant 2M+H ion (15% of the $[M+H]^+$, the field desorption of this compound may involve a bimolecular surface reaction:

$$2M \xrightarrow{-e} [M+H]^+ + [M-H]\cdot$$

The strong hydrogen bonding character of the cytosine may contribute to the formation of a dimer.

Comparison of the f.d. mass spectra of adenine and guanine[34] with their corresponding methylated analogues shows that the introduction of a methyl group causes no difference in f.d.m.s. behaviour except a reduction in BAT by changing the opportunity for hydrogen bonding. The exceptionally low BAT for 1-methyl-uracil is one dramatic example of this effect.

B. Nucleosides

A series of principal nucleosides in DNA and RNA and their methylated analogues have been characterized by their f.d. mass spectra. A list of these compounds together with their f.d. mass spectra are presented in Table 3. In contrast to the corresponding e.i. mass spectra, in which the molecular ion intensity is extremely low or even absent, f.d. mass spectra of all the 24 nucleosides investigated, with the exception of dC·Hcl[†], 5-methylcytidine (5MeC)[†] and 7-methylguanosine (7-MeG)[†] displayed a molecular ion or M+H ion as the base peak. The characteristic sugar (S), (base +H) cleavage products, and ($M-NH_2$) ion for cytidine nucleosides are the only other fragments. The masses of these fragments establish the identity of the base as well as the sugar moiety.

The major fragmentation process in nucleoside spectra involves rupture of the glycosidic bond, leading to either

---

[†]Abbreviation of modified nucleosides are designated as follows:
(1) The first number indicates the position of the substituent. (2) Me=methyl (3) A=adenosine, C=cytidine U=uridine, G=Guanosine, T=Thymidine (4) d=deoxy.

Table 3

Field Desorption Mass Spectra of Some Modified Nucleosides

Compound[b]	e.h.c.[c] (mA)	S	B	B+H	M-17	M	M+H	2M+H	Others[d]
A	19	5%	2%	3%		71%	100%		
1-MeA	20.5	4	1	4		100	25		
N^6-MeA	16					100	56		M+15(7),M+16(6)
dA	17	1				100	44		
1-MedA.HCl	19.5			25			100		
N^6-MedA	15					100	15		
C	19	17		3	2%	12	100	8%	
3-MeC	17.5			25	3		100		
5-MeC	17.5				16		67	100	3M+H(3),2M+S(7)
dC	20.5	57	2	9	11	29	100	3	
dC.HCl	17						3	100	M+B+H(13),2M+S(7)
									2M+B+H(2),3M+H(11)
G	23			3			100	12	M+S(13),2M+S(5),
									$2M-H_2O$(15)
1-MeG	19	11	2	39		100	18		Gua(11)
N^2-MeG	20	4	3	22		100	30	2	
7-MeG	18			100	30		11		
dG	18			3		100	30		
1-MedG	19	3	2			100	40		

cont'd

Table 3 (cont'd)

Compound[b]	e.h.c.[c] (mA)	S	B	B+H	M-17	M	M+H	2M+H	Others[d]
T	19	4		1		100	11		
3-MeT	13					100	12		
U	19	17		5		100	26		m/e 409(5)
3-MeU	16	2	4	2		100	31	1	
5-MeU	15	2		1		100	14		
dU	16	8		4		100	22	2	
5-HmdU	14						100*	55**	

[a]All ions with relative intensity greater than 1% are reported.

[b]Full name of this group of compounds are listed in "Table of Abbreviations".

[c]Emitter heating current (in mA).

[d]Presented in the form: assignment (rel. int.).

*(M+H)+H.

**2(M+H)+H.

the base (B; molecular weight of the free base minus 1), or sugar (S) ion, depending on which half retains the positive charge. This process is schematically shown in Fig. 3.

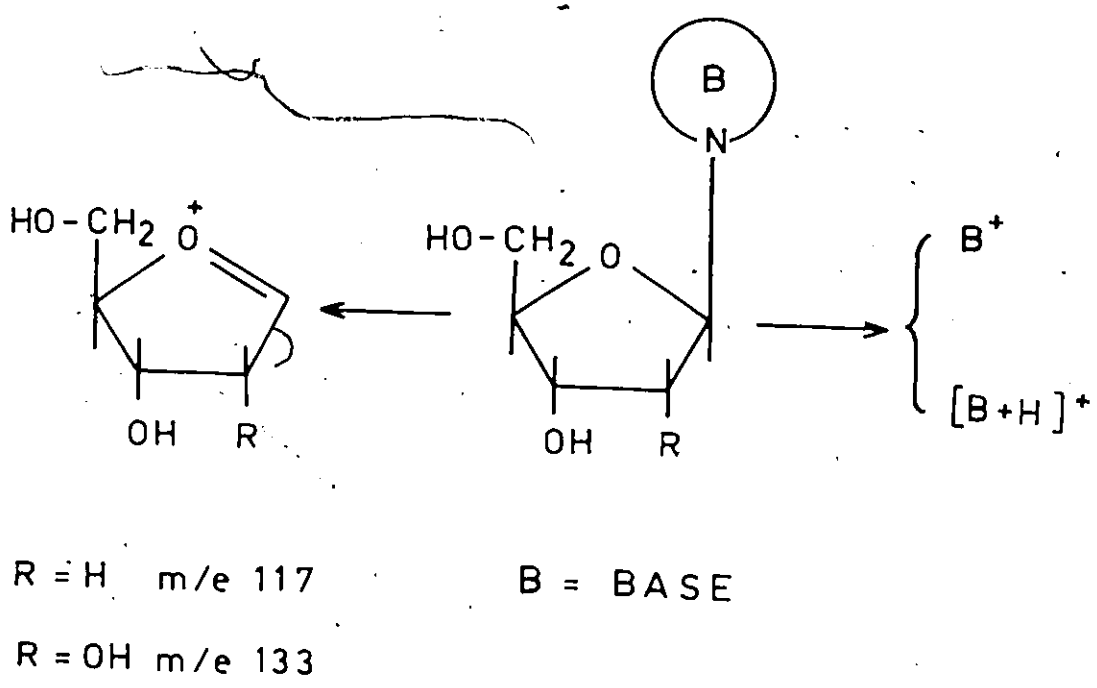


Fig. 3. Fragmentation Scheme of Nucleosides in f.d.m.s.

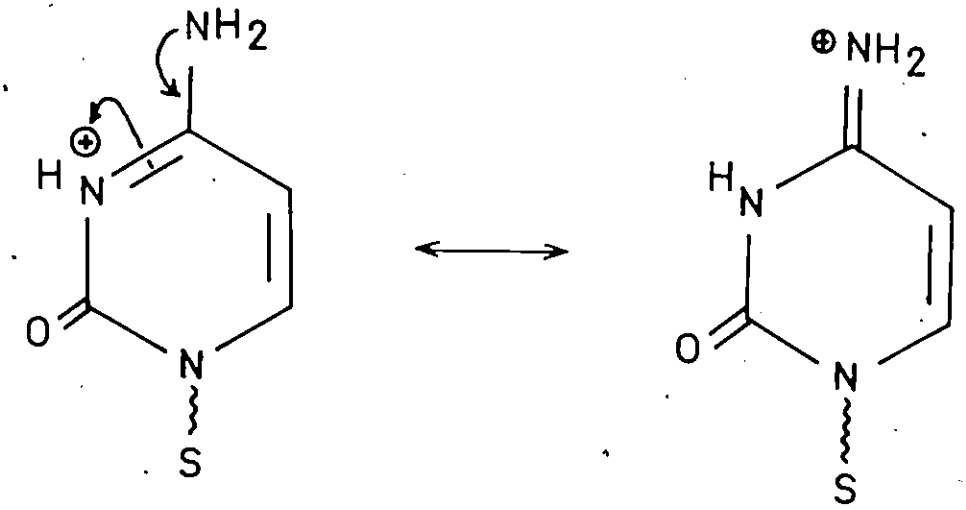
The base ion may exist either as its cationic form or in association with a hydrogen, the latter rearranged primarily from hydroxyl groups of the sugar moiety as judged from the e.i. spectra of O,N-perdeuterio derivatives.[13] The site for attachment of hydrogen is assumed to be one of the hetero-atoms, using its unshared electrons. If traces of the free

base are present as impurities in the nucleoside sample, they will also contribute to the anomalous production of $[B+H]^+$. Comparison of the spectrum of 1-methyl guanosine and 1-methyl-2'-deoxyguanosine under the same anode heating current (19mA) indicates that the abundance of B+H ion may be greater in a riboside than the corresponding 2'-deoxyriboside (39% vs. 3%). Except for the high temperature spectrum of deoxycytidine, other comparisons between adenosine and deoxyadenosine, uridine and deoxyuridine, also agree with this statement. In general, deoxynucleosides normally have a lower BAT than the corresponding ribonucleosides. This difference may be attributed to the stronger hydrogen bonding in the latter compounds.

At least two differences between the nucleoside and its hydrochloride salt were observed. Firstly, the base peak is shifted to the M+H ion. Secondly, protonation of the molecular ion also facilitated glycosidic bond cleavage, favoring formation of the B+H ion. 1-Methyldeoxyadenosine hydrochloride (1-MedA.HCl), for instance, gave a B+H ion of 25% whereas the corresponding ion in 1-methyladenosine (1-MeA) was only 4% even at a higher anode temperature.

Several characteristic features were observed for those nucleosides containing a cytosine skeleton. First of all, a characteristic peak at (M-17) may correspond to either loss of water from the protonated molecular ion or loss of NH_3 from the neutral molecule. A similar ion was

also noted in both e.i. and f.i. mass spectra. High resolution e.i. data of cytidine show that the lost species is an ammonia rather than a hydroxyl radical or water. The same process does not occur in the case of adenosine or 2'-deoxy-adenosine, although both of them possess a free amino group. Another striking feature of cytidine and its analogue is the prominent M+H ion, even at neutral pH. Presumably its stabilized resonance form is responsible for the field-induced proton transfer process.



Finally, cytidine and its analogues have shown their great capacity for forming dimers, trimers, etc. In an extreme case, the 2M+H ion even becomes the base peak in the f.d. mass spectrum of 5-methylcytidine whereas the M+H ion is only 67%. Association is more favorable in an acidic medium

as indicated by the most prominent 2M+H ion (base peak) and the abundant 3M+H (11%) in deoxycytidine hydrochloride.

Among the 5 common nucleosides, guanosine and its analogue are most susceptible to thermal degradation. No signal for the molecular ion of guanosine was observed, either in the e.i. or the f.i. spectrum.[25] Even under f.d. conditions, Schulten and Beckey[33] found that the $[M]^{+\cdot}$ peak was only 20% of the base peak $[B+H]^{+}$. This state of affairs is not surprising, in view of the large number of hydrogen bonding donors and acceptors in this molecule. The thermal energy necessary to mobilize the sample molecules to the ionization sites on the microneedles may also be sufficient (i) to cause thermal degradation prior to f.d. and/or (ii) to produce highly energetic molecular ions which then suffer fragmentation subsequently. In our experiments in which the anode heating current is carefully controlled, it is possible to obtain the M+H ion for guanosine and $M^{+\cdot}$ of 1-methylguanosine (1-MeG) N^2-methylguanosine (N^2-MeG), deoxyguanosine (dG), and 1-methyldeoxyguanosine (1-MedG). Methylation at the 7-position in guanosine represents an unusually difficult situation with regard to producing the maximum intensity of molecular ion. The f.d. mass spectrum (Table 3) shows $[B+H]^{+}$ as the base peak a fact may be attributed to the highly polar betaine structure.[37]

Protonation also facilitates depurination of the molecule as shown in Fig. 4. The second most abundant peak corresponding to (M-17) may arise from loss of NH_3 or loss

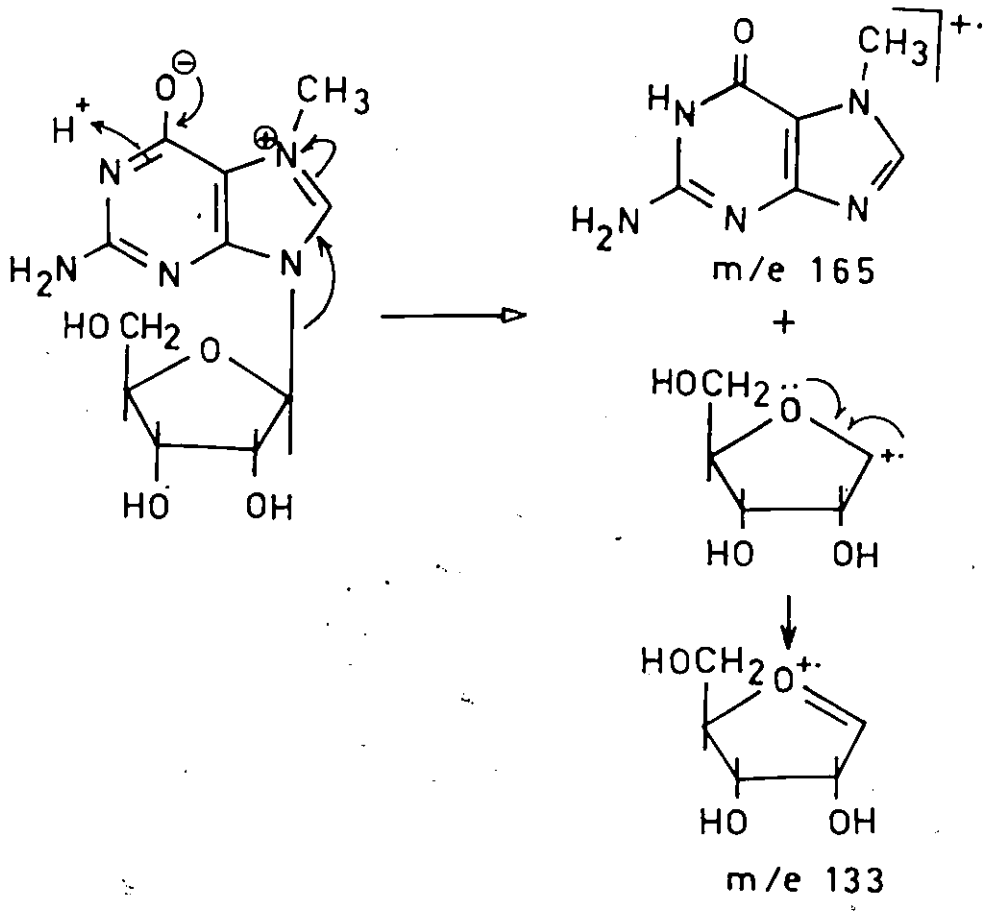


Fig. 4. Fragmentation Scheme of 7-Methylguanosine in f.d.m.s.

of water from the protonated molecular ion. The latter loss ($M-H_2O$) contributes the highest mass peak observed in e.i. mass spectrum. Nevertheless, the $[M+H]^+$ of 11% relative intensity provides a valuable means to identify the unprotected molecule whereas the other methods fail to show this ion.

An equimolar mixture of the authentic nucleosides; adenosine, cytidine, guanosine, uridine and thymidine, was submitted to f.d.m.s. analysis in order to (i) study possible

interactions between the nucleosides (such as the possibility of detecting G-C, A-U or A-T pairs) and (ii) test the applicability of this analytical technique in the identification of nucleic acid components in DNA or RNA hydrolysates. The results which indicate the variation of the relative intensities of each constituent with anode heating current are listed in Table 4. This is an example illustrating how each component in an organic mixture is fractionally desorbed according to its BAT. Thymidine, belonging to the deoxyribonucleoside category, desorbed at the lowest anode temperature whereas guanosine, believed to be the most nonvolatile nucleoside, was the compound to be desorbed at the highest temperature.

Problems arise in the identification of uridine and cytidine. Their molecular weights differ by a single mass unit. Protonation of cytidine, which has been shown (c.f. Table 3) to be a dominant process in its f.d. mass spectrum, produces an ion with the same mass number as the molecular ion of uridine making identification of these components very difficult. Perhaps the occurence of $[2M+H]^+$ (m/e 480) and $[B+H]^+$ (m/e 111) of cytidine may be used to indicate the presence of this compound. After correcting for the ^{13}C isotope contribution, the relative intensities of $[M+H]^+$ of uridine may also provide a hint for its existence. Based on these assumptions, it seems likely that the five nucleosides under investigation fractionally desorbed in the order

Table 4

Relative Intensities of Each Constituent in an Equimolar Mixture of A, G, C, U and T with Different Anode Heating Currents

Ions[a]	m/e	Relative Intensities (%)[b,c]						
		18 mA[d]	19 mA[d]	19.5 mA[d]	20 mA[d]	21 mA[d]	22 mA[d]	23 mA[d]
dS	117	1	1	3				
S	133		1	6	13	12	4	
Cyt	111					1		
Ura	112							
Thy	126			1				
Ade	135							
Gua-H	151					1	21	3
Gua	152						2	1
T	242	100	100	100	9			
C,T+H	243		1	4	11	21		
U,C+H	244	11	17	63	95	100	19	
U+H	245		1	9	14			
A	267	5	12	64	100			
A+H	268		3	19	61	1		
G	283						100	100
G+H	284						23	27
2C+H	487						8	

Cont'd

Table 4 (cont'd)

[a]The full name of this group of ions is listed in "Table of Abbreviations".

[b]Relative intensities are expressed as per cent of the base peak = 100%.

[c]Plotting threshold = 1%.

[d]Anode heating current (mA) at which the spectrum was obtained.

[e]Relative intensities listed have been corrected for the ^{13}C isotope contribution.

T > U > A > C > G which is parallel to that of volatility.

It is also interesting to notice that, at neutral pH, none of the base pairs, even the normally occurring A-U, A-T and G-C pairs, were detected.

C. Nucleotides

The ever-present problems of volatility and thermal stability become even more serious in the case of nucleotides. It has been shown that none of the intact nucleotides is accessible to e.i., f.i. or c.i. mass spectrometric investigation without conversion to volatile derivatives. The work of Schulten and Beckey,[34] has shown that f.d.m.s. is capable of handling this class of compounds.

Six nucleotides, including 3 ribonucleotides, 2 deoxyribonucleotides and 1 cyclic nucleotide were investigated. Their f.d. mass spectra are listed in Table 5. Comparison of the f.d. mass spectra of these compounds permits the elucidation of the following structurally relevant information.

(1) Under optimum conditions, nucleotides with the exception of 5'-GMP and 3'(2")-cyclic AMP gave $[M+H]^+$ as base peak.

(2) In analogy with the corresponding nucleosides, a bond cleavage of the glycosidic linkage gives rise to the (B+H) or (B+2H) ion, thus providing structural information about the base.

(3) The complementary part (M-B) and its dehydrated ion ($M-B-H_2O$) were observed only in 5'-GMP and 5'-CMP.

Table 5

Field Desorption Mass Spectra of Nucleotides[a]

Compound	5'AMP		3'(2')-AMP	5'-CMP	5'dCMP	5'TMP	5'GMP
e.h.c. (mA)[b]	17mA	17mA	18mA	17.5mA	16.5mA	14-16mA	18mA
H_3PO_4+H					99(1)	99(2)	99(96)
Base+H		135(7)		111(11)			151(100)
Base+2H	136(29%)				112(42)		152(69)
N+H	268(7)	268(3)		244(31)	228(1)		
M-17(NH_3 or H_2O-H)	330(1)			306(2)	290(3)	305(3)	
M+H	348(100)	330(6)		324(100)	308(100)	323(100)	364(4)
M+H+H_2O		348(100)		342(1)			
NDP+H-H_2O			*410(6)	386(21)			
NDP+H	428(5)		428(65)	404(17)	388(4)	403(2)	456(6)
M+H_3PO_4+H			446(25)	422(6)	406(11)	412(15)	
NTP+H			508(2)	484(2)			
NDP+H_3PO_4+H				502(1)	486(2)		
2M+H				648(1)	616(8)	646(18)	

cont'd

Table 5 (cont'd)

Compound	5'AMP	3'(2')-AMP		5'-CMP	5'dCMP	5'TMP	5'-GMP
e.h.c. (mA)[b]	17mA	17mA	18mA	17.5mA	16.5mA	14-16mA	18mA
	NDP+S(3)	M+S(36)		M-97-18(8)			M-B-H_2O(9)
		M+S+H_2O(100)		M-97(26)			M-B(6)
OTHER		M+195(6)		2(B+H)+H(3)			M-97-18(6)
IONS		M+123(19)		M+15(10)			98(11)
		M+231(6)		M+B+2H(25)			145(10)
		*3'(2')-ADP					166(9)

[a]Entries are in the form of:- m/e (% rel. int.).

[b]Emitter heating current at which the spectrum was obtained.

(4) A characteristic and abundant ion commonly found in nucleotide spectra which occurs 81 mass units lower than the mass of the $[M+H]^+$ is readily assigned to the protonated nucleoside of the corresponding nucleotide.

(5) Loss of the phosphate moiety (M-97) is particularly prominent in the spectrum of 5'-CMP.

(6) The dehydration species of this ion was also observed in CMP and GMP.

(7) A minor peak which is often observed 17 mass units lower than the molecular weight of the nucleotide may arise from loss of NH_3 from the molecular ion or loss of water from $[M+H]^+$. The origin of the lost species is still not yet established.

(8) Formation of nucleotide diphosphate and triphosphate is another prevalent feature in the f.d. mass spectra of nucleotides. This field-induced phosphorylation process has been observed in many organo-monophosphates as discussed earlier in Part I of this dissertation (P. 101).

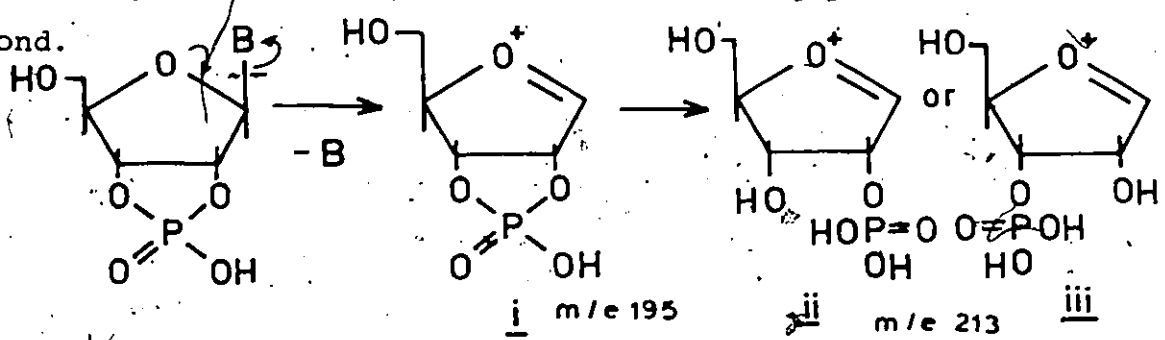
(9) A distinct signal at m/e 99 due to protonated orthophosphoric acid was also detected.

(10) Ion clusters corresponding to the association of the molecular ion and NDP with protonated phosphoric acid were seen. This feature has been reported in other systems.[39]

(11) Regarding formation of a dimer, it is noteworthy that pyrimidine nucleotides seems to be more likely to generate a $[2M+H]^+$ ion than purine nucleotides.

(12) The f.d. mass spectrum of 3'(2')-AMP at 17 mA heating current parallels that of other nucleotides, except the most abundant ion is $[M+19]^+$. This ion may arise from hydrolysis products 3'-AMP and/or 2'-AMP rather than a hydrated ion since it is well known that 3'(2')-AMP is highly susceptible to hydrolysis.

(13) At 18 mA, 3'(2')-AMP behaves in a distinct manner. The f.d. mass spectra of 3'(2')-AMP at 17 mA and 18 mA are shown in Fig. 5(a) and (b) for comparison. The base peak in the higher temperature spectrum is shifted to m/e 480 which corresponds to $[M+S+H_2O]^+$, whereas the [M+H] and [M+19] peaks are totally absent. Another prominent peak at m/e 428 (65%) may be attributed to 3'- or 2'-ADP which may be the hydrolysis products of 3'(2')-ADP at m/e 410 (6%). Other ions are identified as follows: m/e 446 ($M+H_3PO_4+H$), m/e 462 (M+S), m/e 508 (ATP+H), m/e 524 (M+195), m/e 542 (M+213), m/e 560 ($M+213+H_2O$). The charged species with masses m/e 195 and 213 may have the structure i and ii or iii which presumably result from the cleavage of the glycosidic bond.



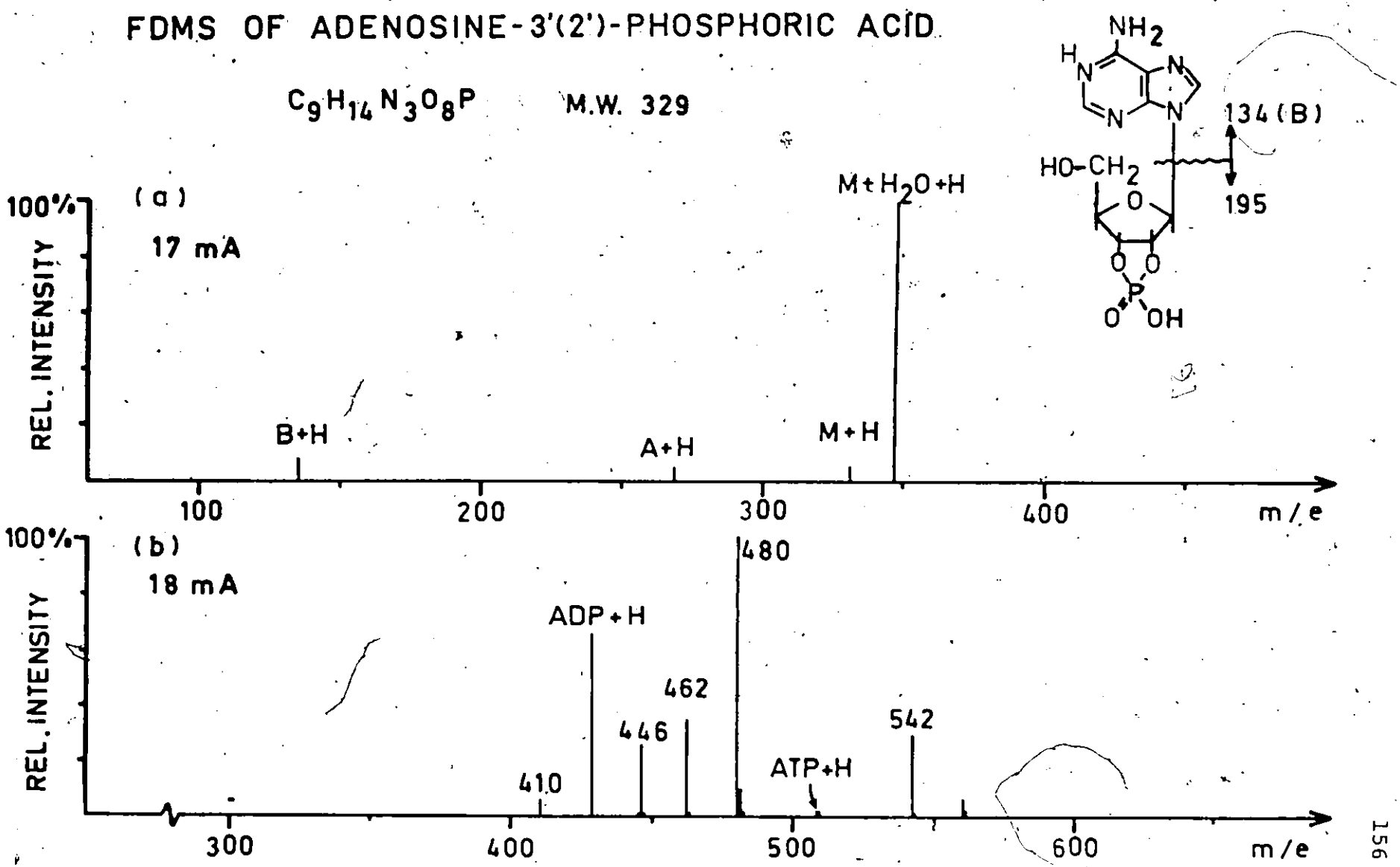


Fig. 5. Field Desorption Mass Spectra of Adenosine-3'(2')-phosphoric Acid (a) at 17 mA and (b) at 18 mA.

(14) Exceptionally strong intermolecular forces between GMP molecules is perhaps the major cause for the low $[M+H]^+$ (6%) and very intense $[B+H]^+$ (base peak, 100%) in the f.d. mass spectra. The presence of abundant protonated phosphoric acid may also induce the glycosidic bond rupture leading to the elimination of a base as a salt occurred in the pyrolysis of nucleic acids.[40]

D. Analysis of DNA Hydrolysates:

The applicability of f.d.m.s. in identification of nucleic acid components in DNA hydrolysate has been tested. Several attempts at detection of major deoxynucleosides have been made on three DNA hydrolysate fractions. Samples supplied by Dr. D.E. Schmidt, Jr. were obtained by enzymatic hydrolysis of calf thymus DNA and then followed by chromatographic separation of Sephadex LH-20 columns.[41] The first sample (Peak I) was repeatedly subjected to f.d.m.s. analysis without success. Virtually no signal other than Na^+ and K^+ was detected. Apparently, removal of such inorganic impurities is necessary before actual analysis can be undertaken. Chromatography of the sample on several ion exchange columns including AG 50W-X8, AG 501-X8, AG 11A8 and Amberlite IR-120H, has been carried out, but none of them reduces the sodium content sufficiently. It has been shown that solvent extraction using a macrocyclic ligand, such as crown ether or cryptand, removes the deleterious effect of metal ions by complex formation. After the sample (Peak I) was extracted

twice with 1.0×10^{-2} M dibenzo-18-crown-6 either in chloroform, it was again submitted to f.d.m.s. analysis. The Na content was reduced at least to a tolerable level and a very intense peak (100%) was recorded at m/e 122 which was finally identified as the (TRIS+H) (TRIS, Tris(hydroxymethyl)-aminomethane) arising from the buffer system. Accordingly, other ions are readily assigned as m/e 241 (4%, 2(TRIS)-H); m/e 277 (10%, 2(TRIS)+^{35}Cl) and m/e 279 (6%, 2(TRIS)+^{37}Cl). No ion corresponding to any deoxynucleosides or bases was observed. Following the same extraction procedure, the second (Peak II) and third (Peak III) fractions were analyzed with the results presented in Tables 6 and 7. As indicated in Table 6, thymidine and deoxyadenosine as well as trace amount of crown ether and its sodium complex are readily identified in the second fraction, whereas deoxyguauosine is the only nuceloside detected in the third fraction. Deoxycytidine is absent in any of the three fractions analyzed indicating it was lost somewhere along the line of chromatographic procedures.

Based on these results, it can be concluded that a detection of modified nucleosides in carcinogen bound DNA by f.d.m.s. is feasible. Detection of minor components will require a more efficient method of sample transfer to the field anode, such as direct addition from a microsyringe.

Table 6

F.d.m.s. Analysis of DNA Hydrolysate: Peak II[a]
(Anode heating current = 17 mA)

m/e	rel. int. (%)	Assignment	m/e	rel. int. (%)	Assignment
18	4	H_2O	251	64	dA[b]
117	5	dS[b]	252	1	
126	2	Thy[b]	360	5	crown ether[b]
242	100	T[b]	383	3	crown ether +Na
243	2	T+H			

[a] Ions with relative intensity greater than 3% are reported.
[b] dS = deoxyribose, Thy = thymine, T = thymidine, dA = deoxyadenosine, crown ether = dibenzo-18-crown-6-ether.

Table 7

F.d.m.s. Analysis of DNA Hydrolysate: Peak III[a]
(Anode heating current = 20 mA)

m/e	rel. int. (%)	Assignment	m/e	rel. int. (%)	Assignment
23	2×10^3 %	Na	476	8	
39	49	^{39}K	487	3	
41	2	^{41}K	491	3	
152	2	Gua[b]+H	522	3	
267	3	dG[b]	525	4	
290	100	dG+Na	526	3	
291	16		537	3	
306	6		582	3	
312	2				
360	8	crown ether[b]			
383	7	crown ether +Na			
384	11				
385	3				
399	4	crown ether+K			

[a]Ion with relative intensity greater than 3% are reported.

[b]Gua = Guanine, dG = deoxyguanosine, crown ether = dibenzo-18-Crown-6-ether.

CHAPTER IV

CONCLUSIONS

The above experiments reveal that the problem of volatility which is the major limitation in the application of mass spectrometry in nucleic acid chemistry can be overcome by means of f.d.m.s. Under careful control of anode heating current, most of the pyrimidine and purine bases, nucleosides and nucleotides, and their methylated derivatives exhibit a prominent $M^{+\cdot}$ or $[M+H]^{+}$. Field desorption mass spectrometry, therefore, shows promise in characterizing normal and modified components in partially fractionated nucleic acid hydrolysates. The method may be particularly useful for study of the interaction between various carcinogenic and mutagenic agents or their active metabolites and nucleic acids, both DNA and RNA. For problems involving structural modification, identification based on nucleosides is preferred, mainly because of the complexity of f.d. spectra of nucleotides. The interfacing of column liquid chromatography with the f.d. mass spectrometer could be beneficial because the emitter wire can be dipped directly into the column eluates and then subsequencely analyzed mass spectrometrically. The utility of the combination has already been shown in studies of steroids[42], vitamins[43], natural porphyrins and chlorophyll

derivatives.[44] However, f.d.m.s. suffers two major drawbacks: (1) lack of fragmentation resulting in inaccessibility of stereochemical details, (2) high sensitivity of f.d. mass spectra to alkali metal contamination.

Work in progress is directed toward further development of the desalting technique, as well as the application of f.d.m.s. in the actual analysis of modified DNA hydrolysates.

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APPENDIX I

Field Desorption Mass Spectra of Phosphatidyl Cholines[a,b,c]:

I. Dibutroyl PC R_1[c]$=R_2=(CH_2)_2CH_3$ e.h.c.[†]=18 mA

m/e 87 (11%)*, 104 (7), 184 (2), 242 (8, $[M+87]^{++}$), 242.5 (2), 269 (13), 270 (2), 310 (5, M-RCOO[d]), 311 (3), 312 (12, M-RCOO+2H or phosphatidic acid), 313 (2), 349 (5), 398 (100, M+H), 399 (22), 400 (4), 412 (1, $M+CH_3$) 413 (2), 441 (4, $[2M+88]^{2+}$), 441.5 (3), 483 (3, M+86), 501 (6, M+ choline), 502 (3), 537 (3, M+140), 563 (17, M+166), 564 (5), 581 (5,M+184), 582 (2), 666 (3, M+269), 709 (8, M+312) 795 (33, 2M+H), 796 (13).

II. Dioctanoyl PC $R_1=R_2=(CH_2)_6CH_3$ e.h.c.=18 mA

m/e 86 (1%), 88 (51), 104 (74), 105 (5), 146 (5), 184 (17), 185 (5), 198 (2), 228 (11), 229 (3), 230 (203, RCOO+87), 231 (44), 232 (7), 258 (7), 366 (4, M-RCOO), 367 (3), 368 (41, M-RCOO+2H), 369 (11), 384 (34, M-RCO+2H), 385 (87), 508 (6, M-H), 510 (100, M+H), 511 (29), 512 (5), 598 (3, M+88), 613 (7, M+choline), 693 (6, M+184), 694 (3), 739 (8, M+230 (RCOO+87)), 740 (3), 893 (3, M+384).

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*m/e value (rel. int. %), in some cases an assignment follows the rel. int.

[†]e.h.c. = emitter heating current.

III[d] Dicaproyl PC $R_1=R_2=(CH_2)_8CH_3$ e.h.c.=21 mA

m/e 86 (5%), 88 (3), 104 (3), 128 (3), 129 (31), 130 (4), 269 (44), 270 (9), 326 (3, $[M+87]^{++}$), 327 (3), 382 (4, M-phC-H), 383 (4, M-phC), 394 (16, M-RCOO), 396 (7, (M-RCOO+2H), 566 (100, M+H), 567 (17), 568 (12), 580 (13, $M+CH_3$), 581 (5), 588 (19, M+Na), 589 (6), 610 (8, M+45), 611 (5), 651 (26, M+86), 652 (11), 653 (4), 669 (28, M+choline), 670 (8), 671 (3).

IV Dilauroyl PC $R_1=R_2=(CH_2)_{10}CH_3$ e.h.c.=20 mA

m/e 86 (6%), 88 (2), 104 (15), 184 (4), 269 (5), 298 (2, RCOO+99), 349 (4), 354 (2, $[M+87]^{++}$), 422 (3,M-RCOO), 424 (8, M-RCOO+2H), 425 (3), 440 (1, M-phC+H), 620 (3, (M-H), 621 (2,M), 622 (100, M+H), 623 (40), 624 (8), 636 (3, $M+CH_3$), 707 (3, M+86), 725 (6, M+choline), 726 (2), 787 (1, M+166), 805 (5, M+184), 806 (2).

V Dimyristoyl PC $R_1=R_2(CH_2)_{12}CH_3$ e.h.c.=19 mA

m/e 86 (12%), 88 (10), 104 (53), 105 (4), 129 (1), 130 (4), 184 (6), 198 (2), 269 (13), 270 (2), 314 (2, RCOO+87), 362 (2, RCOO+99), 349 (4), 450 (3, M-RCOO), 451 (2), 452 (9, M-RCOO+2H), 453 (2), 650 (4, dimyristyl phosphatidyl-N-methyl ethanolamine, $M-2CH_3+2H+H$), 676 (3, M-H), 677 (2,M), 678 (100, M+H), 679 (40), 680 (10), 681 (2), 692 (4, $M+CH_3$), 693 (3), 700 (8, M+Na), 701 (2), 722 (3, M+45), 735 (2, M+58), 749 (3, M+72), 763 (4, M+86), 764 (2), 765 (3, M+88), 781 (13, M+choline), 782 (7), 783 (3), 861 (5, M+184), 862 (2), 946 (5, M+269), 947 (3).

VII[f§] 1-Palmitoyl-2-myristoyl PC $R_1=(CH_2)_{14}CH_3$

$R_2=(CH_2)_{10}CH_3$ e.h.c.=23 mA

m/e 86 (11), 104 (8), 124 (15), 125 (5), 129 (15), 130 (3), 211 (15, R_2CO), 212 (3), 239 (5, R_1CO), 269 (65), 270 (9), 271 (5), 314 (5, $R_2COO+87$), 315 (5), 342 (8, $R_1COO+87$), 343 (3), 450 (5, $M-R_1COO$), 452 (3, $M-R_1COO+2H$), 478 (3, $M-R_2COO$), 479 (3), 480 (4, $M-R_2COO+2H$), 522 (99, M-phC-H), 523 (125, M-phC), 524 (49), 525 (12), 551 (4), 552 (4), 692 (8, $M+H-CH_3$), 693 (6), 694 (3), 706 (100, M+H), 707 (45), 708 (18), 709 (4), 721 (8, $M+CH_3$), 722 (10), 723 (4), 728 (8, M+Na), 729 (5), 761 (15, M+56), 762 (3), 763 (5, M+58), 764 (3), 791 (5, M+86), 809 (5, M+104)

VIII[h] 1-Palmitoyl-2-oleoyl-PC $R_1=(CH_2)_{14}CH_3$

$R_2=(CH_2)_7CH=CH(CH_2)_7CH_3$ e.h.c.=22 mA

m/e 86 (10%), 88 (1), 104 (17), 105 (5), 129 (3), 130 (9), 184 (2), 269 (8), 270 (2), 481 (4, $(M-R_2COO+3H?)^+$, 503 (3, $(M-R_1COO-H)?$), 578 (5, M-phC), 723 (6), 724 (3), 746 (4, $M-CH_3+2H$), 758 (6, M-H), 759 (4, $[M]^+$), 760 (100%, M+H), 761 (54), 762 (16), 763 (6), 774 (12, $M+CH_3$), 775 (7), 780 (3), 782 (33, M+Na), 783 (15), 784 (6), 831 (3, M+72), 845 (6, M+86), 846 (5), 847 (3, M+88), 863 (15, M+choline), 864 (11), 865 (3), 888 (3, M+129), 899? (4, M+130), 925? (2, M+166), 943? (2, M+184), 956? (3, $(M+197)?$).

IX[i] 1-Oleoyl-2-palmitoyl PC, $R_1(CH_2)_7CH=CH(CH_2)_7CH_3$, $R_2=(CH_2)_{14}CH_3$ e.h.c.=21 mA

m/e 86 (45%), 87 (6), 88 (3), 104 (47), 105 (2), 129 (8), 130 (9), 152 (4), 184 (4), 198 (4), 199 (3), 269 (78), 270 (17), 271 (3), 349 (4), 423 (3, $[M+87]^{+2}$), 423.5 (1), 451 (3), 451.5 (4), 576 (7, M-phC-H), 577 (7, M-phC), 746 (2, $M-CH_3+2H$), 758 (4, M-H), 759 (2, M), 760 (100, M+H), 761 (60), 761 (18), 763 (4), 774 (17, $M+CH_3$), 775 (8), 776 (6), 782 (8, M+Na), 783 (7), 784 (2), 816? (4, M+57?), 831? (8, M+72), 832? (7), 833? (2), 845? (12, M+86), 846? (3), 847? (2), 864? (19, M+105?), 865? (12), 866? (4), 924? (6, M+165), 925? (3), 944? (3, M+185), 945? (2), 956? (6, M+197), 957? (3).

X[j,k §] 1-Palmitoyl-2-linoleoyl PC $R_1=(CH_2)_{14}CH_3$, $R_2=(CH_2)_7(CH=CHCH_2)_3(CH_2)_3CH_3$ e.h.c.=20 mA

m/e 88 (1%), 104 (6), 130 (24), 342 (78, $R_1COO+87$), 343 (11), 366 (40, $R_2COO+87$), 367 (4), 502 (13, $M-R_1COO$), 503 (2), 526 (2, $M-R_1COO+H+Na$), 778 (3), 780 (100%, M+Na), 781 (39), 782 (9), 861 (1, M+104).

XI Distearoyl PC $R_1=R_2=(CH_2)_{16}CH_3$ e.h.c.=19.5 mA

m/e 86 (43%), 87 (3), 88 (3), 104 (22), 105 (2), 129 (4), 269 (98), 270 (7), 341 (3), 371 (118, RCOO+87), 372 (28), 373 (5), 507 (10, M-RCOO+H), 508 (7), 509 (3), 607 (43, M-phC), 608 (32), 609 (15), 610 (7), 761 (12, $M-2CH_3+2H$), 762 (8), 775 (8, $M-CH_3+H$), 776 (5), 790 (100, M+H), 791

(53), 792 (18), 793 (3), 804 (26, M+CH_3), 805 (18), 806 (7), 819 (3), 833 (3, M+44), 847 (8, M+58), 848 (5), 861 (7, M+72), 862 (7), 875 (18, M+86), 876 (14), 877 (5), 893 (28, M+choline), 894 (18), 895 (8), 916? (7, M+127), 917?(5), 955? (11, M+166), 956? (12), 973? (7, M+184), 974? (3), 986? (15, M+197?), 987?(5).

XII[5] Dioleoyl PC $R_1=R_2=(CH_2)_7CH=CH(CH_2)_7CH_3$

e.h.c.=20 mA

m/e 86 (7%), 88 (5), 104 (34), 105 (2), 129 (3), 130 (2), 184 (3), 269 (13), 270 (2), 368 (142, RCOO+87), 369 (42), 370 (8), 504 (2, M-RCOO), 506 (4, M-RCOO+2H), 507 (2), 603 (11, M-phC), 604 (6), 605 (4), 749 (4), 750 (2), 760 (3), 772 (3, M+H-CH_3+H), 784 (4, M-H), 785 (3, M), 786 (100, M+H), 787 (55), 788 (12), 789 (3), 800 (5, M+CH_3), 801 (3), 808 (16, M+Na), 809 (11), 810 (4), 843 (2, M+58), 857 (3, M+72), 858 (2), 871 (4, M+86), 873 (3, M+88), 889 (11, M+104), 890 (8), 891 (3).

XIII Dilinoleoyl PC, $R_1=R_2=(CH_2)_7(CH=CHCH_2)_2(CH_2)_3CH_3$

e.h.c.=21 mA

m/e 23 (8%, Na^+), 86 (15), 87 (3), 88 (6), 104 (68), 105 (3), 129 (4), 130 (9), 131 (2), 184 (5), 198 (2), 269 (10), 367 (15, RCOO+87+H), 368 (6), 369 (2), 503 (4, M-RCOO+H), 504 (2), 505 (7, M-RCOO+3H), 599 (2, M-phC), 600 (5, M-phC+H), 601 (4), 746 (19), 747 (10), 782 (100, M+H), 783 (5), 784 (20), 785 (6), 796 (5, M+CH_3), 797 (4), 802 (9, M-2H+Na),

803 (6), 804 (86, M+Na), 805 (51), 806 (18), 807 (5), 808 (3), 820 (3, M+K), 867 (3, M+86), 868 (4), 869 (3), 885 (15, M+choline), 886 (8), 887 (5), 910? (2, M+129), 911? (2, M+130).

XIV Dilinolenoyl PC $R_1=R_2=(CH_2)_4(CH=CHCH_2)_3(CH_2)_3CH_3$

e.h.c.=19 mA

m/e 104 (38%), 105 (2), 188 (4), 189 (3), 312 (9), 313 (4), 352 (28), 353 (10), 355 (9), 356 (9), 357 (5), 358 (8), 359 (3), 364 (25, RCOO+87), 365 (9), 366 (3), 368 (4), 369 (3), 370 (9), 371 (4), 372 (5), 373 (3), 384 (4), 385 (3), 386 (7), 387 (3), 392 (5), 393 (3), 394 (9), 755 (10), 756 (7), 757 (7), 758 (3), 759 (8), 778 (100, M+H, base peak), 779 (50), 780 (19), 781 (7), 800 (16, M+Na), 801 (7), 802 (3), 881 (65, M+choline), 882 (39), 883 (17), 884 (7), 885 (9), 886 (6).

XV § Diarachidonyl PC $R_1=R_2=(CH_2)_3(CH=CHCH_2)_4(CH_2)_3CH_3$

e.h.c.=19 mA

m/e 86 (8), 88 (2), 104 (252), 105 (17), 390 (46, RCOO+87), 391 (10), 392 (7), 393 (4), 647 (17, M-phC+H), 648 (9), 649 (4), 745 (7, M-84), 746 (3), 811 (5, M-18), 830 (4, M+H), 852 (22, M+Na), 853 (15), 854 (7), 910 (10), 911 (7), 933 (100, M+104), 934 (64), 935 (29), 936 (12), 937 (5), 950 (7), 951 (4).

XVI Dihexadecyl Ether PC $R'^{1}=R''^{1}=(CH_2)_{15}CH_3$

e.h.c.=21 mA

m/e 86 (6%), 88 (5), 104 (56), 105 (3), 184 (12), 226 (4), 269 (3), 312 (3), 424 (5), 466 (8, M-RO+2H), 622 (5), 647 (17, $M+H-N(CH_3)_3$), 648 (10), 669 (18), 670 (11), 692 (14, $M-CH_3+2H$), 693 (6), 704 (8, M-H), 705 (3), 706 (100, M+H), 707 (56), 708 (20), 720 (6, $M+CH_3$), 721 (5), 728 (13, M+Na), 729 (6), 734 (13, (M+29)), 735 (6), 736 (3), 763 (9, M+58), 764 (5), 791 (5, M+86), 792 (3), 793 (6, M+88), 809 (16, M+104), 810 (9), 811 (3).

XVII 1-Octadec-9-enyl,2-Hexadecyl) ether PC

$R'=(CH_2)_8(CH=CH)(CH_2)_7CH_3$ $R''=(CH_2)_{15}CH_3$ e.h.c.=21 mA

m/e 86 (23%), 88 (9), 104 (46), 105 (3), 129 (5), 130 (5), 184 (2), 198 (2), 269 (20), 270 (4), 284 (2), 312 (2), 326 (5, R"O+87-2H), 349 (6), 410 (2), 411 (3), 645 (3), 673 (6, $M-N(CH_3)_3$), 674 (8, $M+H-N(CH_3)_3$), 675 (5), 677 (3), 679 (8, $XVI-2CH_3+H$), 704 (18, XVI-H), 706 (29, XVI+H), 707 (15), 708 (4), 718 (9, $M-CH_3+2H$), 719 (3), 720 (3), 730 (7, M-H), 732 (3), 732 (100%, M+H), 733 (56), 734 (14), 735 (5), 744 (9, $M-H+CH_3$), 745 (3), 746 (9, $M+CH_3$), 747 (3), 760 (5, M+29), 761 (4), 789 (3, M+58), 803 (3, M+72), 804 (2), 817 (4, M+86), 835 (7, M+104), 836 (4).

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[a]All ions with relative intensity greater than 2% and others of particular interest are reported.

[b]All masses listed here have been corrected for the mass defect. (eg. the actual mass recorded for $C_{32}H_{65}O_8NP$ at m/e 622.5 is labelled as 622).

[c]R=hydrocarbon chain of the fatty acid residues. The subscript refers to the carbon number of the glycerol skeleton where the acyl group is attached.

[d]At 22 mA, m/e 651 (M+86) ion became the base peak (100%), whereas the M+H ion was reduced to 61%. Other ions at m/e 269, 383, 394, 580, 669 raised to 63%, 21%, 26%, 34%, 38%, respectively.

[e]At 21 mA, an ion at m/e 890 (M+269) was also seen (10%). Intensities of other significant ions are: m/e 86 (14%), 88 (2), 104 (30), 184 (7), 269 (25), 349 (10), 422 (6), 424 (10), 636 (6), 707 (7), 725 (15), 787 (3), 805 (7).

[f]In other scan (22.5 mA), associated ions are more predominant: m/e 721 (45%, M+CH_3), 761 (28, M+56), 763 (15, M+58), 777 (10, M+72), 791 (33, M+86), 809 (30, M+104), 834 (13, M+129), 835 (12, M+130), 872 (13, M+166).

[g]This e.h.c. was surely above the BAT, but only at this current range, ions with reasonably high intensity were obtained.

[h]At lower e.h.c., (20-21 mA), the base peak was M+Na . An additional ion corresponding to choline monohydrate (m/e 122)(34% at 20 mA, 3% at 21 mA) was also observed.

[i]In other scans (20.5 mA and 21 mA) m/e 269 is the strongest peak (140% and 137% with respect to M+H as 100%).

[j]Before silicic acid column chromatography, m/e 130 was the base peak and M+130 was the strongest peak around the molecular weight region.

[k]Data was processed by computer.

[l]R'=hydrocarbon chain of the ether PC.

[§]The mass spectrum was normalized according to the largest peak in the molecular weight region.

[†]"?" means uncertainty in the assignment or mass number.

Appendix II

F.d.m.s. of Lysophosphatidyl Cholines[a,b]

XXII Palmitoyl LPC $R=(CH_2)_{14}CH_3$ e.h.c.[†]=18 mA

m/e 85 (2%), 104 (47%), 105 (3), 479 (3, M+2H-H_2O), 496 (100, M+H), 497 (27), 498 (7), 518 (13, M+Na), 519 (4), 534 (3, M+K), 581 (2, M+86), 599 (41, M+choline), 600 (15), 601 (3), 679 (16, M+184), 680 (6), 694 (3, M+199).

XXIII Stearoyl LPC $R=(CH_2)_{16}CH_3$ e.h.c.=18 mA

m/e 104 (51%), 184 (7), 242 (3, M-RCOO+2H), 479 (3), 496 (7)[c], 498 (4), 506 (18, M+H-H_2O), 507 (6), 508 (2), 522 (5, M-H), 524 (100%, M+H), 525 (34), 526 (7), 527 (3), 547 (18, M+Na+H), 548 (5), 562 (4, M+K), 563 (2), 627 (28, M+choline), 628 (14), 629 (5), 707 (15, M+184).

XXIV Oleoyl LPC $R=(CH_2)_7CH=CH(CH_2)_7CH_3$ e.h.c.=17 mA

m/e 86 (4%), 88 (10), 104 (47), 105 (5), 184 (13), 185 (2), 206 (6, phC-H+Na), 240 (5, M-RCOO), 242 (3, M-RCOO+2H), 258 (51, M-RCO+2H), 259 (7), 280 (39, M-RCO+Na+H), 281 (3), 361 (2, M-RCO+H+choline), 362 (2), 504 (3, M+H-H_2O), 520 (4, M-H), 521 (2,M), 522 (100, M+H), 523 (35), 524 (11), 525 (4), 536 (12, M+CH_3), 537 (2), 544 (87, M+Na), 545 (17), 546 (5), 625 (35, M+choline), 626 (14), 627 (5), 705 (5, M+184), 706 (4), 779 (13, M+258), 780 (6), 781 (3), 801 (35, 2M-RCO+Na+H(M+280)), 802 (11), 803 (5), 883 (11, M+361).

[†] e.h.c. = emitter heating current

XXV[§] Linolenoyl LPC R=$(CH_2)_4(CH{=}CHCH_2)_3(CH_2)_3CH_3$

e.h.c.=17 mA

m/e 86 (6), 88 (3), 104 (134), 105 (9), 160 (2), 184 (8), 198 (3), 206 (3), 240 (5, M-RCOO), 242 (3, M-RCOO+2H), 258 (6, M-RCO+2H), 269 (2), 287 (2, 184-H+choline), 288 (2), 361 (5, M-RCO+H+choline), 364 (5, RCOO+87), 382 (3), 412 (4, M-choline)[d], 428 (6), 468 (3), 496 (6), 500 (12, M+H-H_2O), 501 (4), 502 (2), 518 (100, M+H), 519 (30), 520 (11), 534 (5, M+H_2O-H), 540 (46, M+Na), 541 (22), 542 (7), 621 (67, M+104), 622 (22), 623 (10), 624 (3), 701 (13, M+184), 702 (5), 703 (5).

XXVI Arachidonyl LPC[e] R=$(CH_2)_3(CH{=}CHCH_2)_4(CH_2)_3CH_3$

e.h.c.=11 mA

m/e 104 (82%), 105 (5), 106 (3), 182 (7), 184 (9), 258 (37, M-RCO+2H), 259 (5), 280 (4, 258-H+Na), 281 (3), 282 (4), 544 (100, M+H), 545 (33), 546 (12), 547 (4), 566 (11, M+Na), 567 (3), 647 (7, M+104), 648 (3), 801 (5, M+258), 802 (3).

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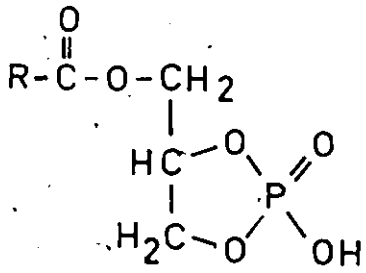
[a]All ions with relative intensity greater than 3% and others of particular interest are reported.

[b]All masses listed have been corrected for the mass defect.

[c][Palmitoyl LPC+H]$^+$.

[d]At 16 mA, an additional ion at m/e 414 (10%) was also observed. (p. 176).

[§]The mass spectrum was normalized according to the largest peak in the molecular weight region.



m/e 414

[e]At 17 mA, the base peak shifted to m/e 647 (M+104) whereas at 20 mA, the base peak became m/e 390 (RCOO+87).

Appendix III

Field Desorption Mass Spectra of Some Phosphatidyl Ethanolamines[a,b]

XXVII Dilauroyl PE $R'=R''=(CH_2)_{10}CH_3$ e.h.c.= 18 mA

m/e 124 (2%), 183 (2,phE+43), 226 (3, RCO+43), 380 (4, M-RCOO), 382 (6, M-RCOO+2H), 439 (4, M-phE), 457 (3, dilauroyl glycerol +H), 479 (3, m/e 457-H+Na), 562 (1, $M-NH_3$), 563 (1), 580 (100, M+H), 581 (35), 582 (8), 583 (2), 584 (4), 602 (5, M+Na), 603 (3), 760 (2).

XXVIII Dipalmitoyl PE $R'=R''=(CH_2)_{14}CH_3$ e.h.c.=18 mA

m/e 124 (1%), 282 (3, RCOO+43), 393 (3), 436 (6, M-RCOO), 437 (2), 438 (4, M-RCOO+2H), 550 (2), 551 (12, M-phE), 552 (7), 553 (2), 568 (8, dipalmitoyl glycerol+H), 569 (4), 674 (5, $M-NH_3$), 675 (2), (2,M), 692 (100, M+H), 693 (37), 694 (10), 695 (2), 696 (2), 714 (2, M+Na).

XXIX Distearoyl PE $R'=R''=(CH_2)_{16}CH_3$ e.h.c.=18 mA

m/e 310 (18, RCO+43), 464 (32, M-RCOO), 465 (13), 466 (9, M-RCOO+2H), 467 (5), 625 (12, M-140+2H), 626 (5), 730 (21, $M-NH_3$), 731 (9), 732 (7), 733 (3), 748 (100, M+H), 749 (45), 750 (9), 751 (7).

XXX Dioleoyl PE $R'=R''=(CH_2)_7CH=CH(CH_2)_7CH_3$ e.h.c.=19 mA

m/e 123 (6), 308 (6, RCO+43), 461 (6), 462 (16, M-RCOO), 463 (6), 464 (10, M-RCOO+2H), 465 (4), 603 (M-phE), 604 (3), 620 (3), 621 (11, dioleoyl glycerol+H), 622 (8), 623 (3), 688 (8), 689 (3), 690 (14), 692 (10), 698 (3),

716 (8, M-27), 717 (7), 718 (7), 719 (3), 726 (6, M-NH_3), 727 (3), 742 (3, M-H), 743 (3), 744 (100, M+H), 745 (59), 746 (40), 747 (12), 748 (4).

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[a]All ions with relative intensity greater than 2% are reported.

[b]All masses listed are nominal masses and have been corrected for mass defect.

Appendix IV

Field Desorption Mass Spectrum of Some Lysophosphatidyl Ethanolamines[a]

XXXI Palmitoyl LPE $R-(CH_2)_{14}CH_3$ e.h.c.=18 mA

m/e 142 (16%, phE+H), 156 (2, phosphoryl ethanolamine methyl ester+H), 200 (10, M-RCOO+2H), 216 (6, glycerophosphoryl ethanolamine+H), 282 (3, RCO+43), 425 (2), 434 (2, $M-H_2O-H$), 437 (2, $M+H-NH_3$), 452 (3, M-H), 453 (3,M), 454 (100, M+H), 455 (45), 456 (6), 477 (3, M+Na+H).

XXXII Oleoyl LPE $R=(CH_2)_7CH=CH(CH_2)_7CH_3$ e.h.c.=18 mA

m/e 62 (10%), 142 (9, phE+H), 143 (2), 200 (37, M-RCOO+2H), 201 (4), 216 (6, glycerophosphoryl ethanolamine+H), 217 (2), 283 (3, RCOO+2H), 308 (3, RCO+43), 419 (4), 426 (6), 452 (9)[b], 454 (12)[c], 478 (6, M-H), 479 (2,M), 480 (100, M+H), 481 (27), 482 (11), 483 (3), 621 (5, M+142), 681 (5, M+200).

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[a] All ions with relative intensity greater than 2% are reported.

[b] $[Palmitoyl\ LPE-H]^+$ (derived from impurities).

[c] $[Palmitoyl\ LPE+H]^+$ (derived from impurities).

Appendix V

Field Desorption Mass Spectra of Some Phosphatidic Acids and Lysophosphatidic Acids[a]

XXXV[b §] Dipalmitoyl PA M.W. 648.5 e.h.c.=14 mA m/e 86 (164%), 87 (10), 88 (24), 99 (8, H_3PO_4+H), 100 (10), 116 (65, $H_3PO_4 \cdot H_2O$), 292 (15), 294 (31), 393 (39, M-RCOO), 394 (10), 395 (43, M-RCOO+2H), 396 (13), 407 (16), 410 (41, palmitoyl LPA)[c], 411 (23)[c], 412 (31, Palmitoyl LPA+2H)[c], 467 (10), 469 (29), 470 (18), 478 (24), 479 (11), 480 (14), 492 (13), 494 (19), 496 (10), 497 (10), 508 (10), 538 (18, dipalmitoyl glycerol), 552 (14, M-phosphoric acid+H), 553 (11), 554 (11), 555 (20), 556 (10), 630 (11, M-H_2O), 647 (11, M-H), 649 (100, M+H), 650 (55), 651 (16), 664 (10, M-H+CH_3), 666 (118, M+H_2O), 667 (40), 668 (13), 669 (5), 671 (20, M+Na), 734 (85, dipalmitoyl PC-H+CH_3)[c], 735 (28)[c], 736 (21)[c], 737 (15)[c], 747 (35, dipalmitoyl PC-H+CH_3)[c], 748 (15)[c], 761 (23, dipalmitoyl PC-H+CH_2CH_3)[c], 762 (13), 802 (14), 803 (12), 804 (10), 869 (14), 871 (11), 882 (14), 883 (11).

XXXVI Caproyl LPA M.W. 326.3 e.h.c. = 10 mA m/e 80 (15%), 99 (3, H_3PO_4+H), 116 (5, $H_3PO_4 \cdot H_2O$), 173 (16, glycerophosphoric acid+H), 189 (17, RCOO+H_2O), 206 (10), 308 (6, M-H_2O), 309 (M+H-H_2O), 327 (100, M+H, base peak 328 (15), 341 (8, M+CH_3), 344 (17, M+H_2O), 349 (3, M+Na),

355 (10, M+CH_2CH_3), 377 (10), 425 (14, (M+H)·H_3PO_4), 453 (6), 481 (9, M+155), 499 (10, M+173), 501 (5), 515 (21, M+RCOO+H_2O), 516 (5), 527 (5), 635 (7, 2M+H-H_2O), 653 (31, 2M+H), 654 (9), 670 (3, 2M+H_2O), 681 (7), 807 (9, 2M+155), 825 (3, 2M+173).

XXXVII Dipalmitoyl LPA M.W. 410.3 e.h.c.=12 mA

m/e 170 (5%), 172 (7, glycerophosphoric acid), 255 (3, RCOO), 393 (10, M+H-H_2O), 411 (100, M+H, base peak), 412 (46), 413 (10), 416 (8), 429 (45, M+H+H_2O), 430 (9), 470 (10), 491 (13, palmitoyl lysopyrophosphoric acid+H), 509 (10, M+H+H_3PO_4), 786 (7), 802 (7), 803 (22, 2M+H-H_2O), 804 (8), 820 (9, 2M), 821 (14, 2M+H), 822 (6).

XXXVIII Oleoyl Lysophosphoric Acid M.W. 436.3 e.h.c.=10 mA

m/e 172 (1%), 190 (6%), 300 (23, RCOO+H_2O), 437 (30, M+H), 438 (13), 454 (100, M+H_2O, base peak), 455 (21), 456 (8), 459 (5, M+Na), 535 (5, M+H+H_3PO_4), 552 (4, (M+H)+H_3PO_4+H_2O), 609 (3, M+173), 610 (7), 873 (4, 2M+H), 890 (4, 2M+H_2O).

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[a] Except 31, all ions with relative intensity greater than 5% or others of particular interest are reported. All masses are nominal mass and corrected for mass defect.

[b] Plotting threshold = 10%.

[c] Ions arose from impurities.

[§] The mass spectrum was normalized according to the largest peak in the molecular weight region.

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