Intracytoplasmic Membrane Assembly in Phototrophically Grown Cultures of *Rhodopseudomonas sphaeroides*

by

Kenneth Walter Koeritz, Jr.

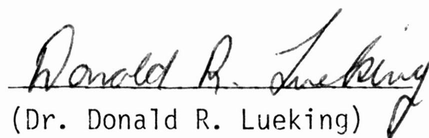
Biology Department

Submitted in Partial Fulfillment of the Requirement of the

University Undergraduate Fellows Program

1980 - 1981

Approved by:



(Dr. Donald R. Lueking)

April 1981

Abstract

The differential synthesis of intracytoplasmic and whole cell phospholipids has been monitored in synchronously and asynchronously dividing cultures of *Rhodopseudomonas sphaeroides* by employing a non-specific deuterium labeling technique. Following the phototrophic growth of cells of *R. sphaeroides* in D_2O-based medium, mass spectrometric analysis of purified methyl esters of *cis*-vaccenate (the predominant (90%) fatty acid possessed by *R. sphaeroides*) revealed an average level of deuteration of 23 atoms of deuterium incorporated per fatty acid molecule. This level of acyl group deuteration was calculated to contribute over 70% of the increased specific density of membranes obtained from cells grown in deuterated medium. The deuterated and protonated species of methyl vaccenate were found to be separable by gas-chromatography, and by monitoring the decrease in the relative quantity of deuterated vaccentate to normally protonated vaccenic acid during a D_2O to H_2O growth transition, the production of cellular fatty acids could be monitored. This latter technique was employed to examine the pattern of membrane phospholipid production in cells of *R. sphaeroides*.

ACKNOWLEDGEMENTS

I would like to thank Dr. Donald R. Lueking for his ideas, advice, and encouragement throughout this endeavor. The knowledge I have gained has made the effort truly rewarding. Also, I would like to thank Dr. Karl Dahm of the Institute of Developmental Biology for his expertise in the GC-Mass spectrometer analysis and separation of deuterated and non-deuterated species of methyl-vaccenate.

TABLE OF CONTENTS

Page

I. INTRODUCTION 1

II. MATERIALS AND METHODS. 5

1. Bacterial strain, media, and conditions of growth. 5
2. Culture synchronization procedure. 6
3. Cell enumeration 6
4. Intracytoplasmic membrane purification 6
5. Phospholipid extraction. 7
6. Preparation of fatty acid methyl esters. 8
7. Purification of fatty acid methyl esters 8
8. Gas-chromatographic resolution of deuterated and protonated species of _cis_-vaccenyl methyl esters. 9
9. Mass spectrometric analysis of deuterated methyl vaccenate 9

III. RESULTS. 10

1. The increased molecular weight of deuterated vaccenate and its contribution to increased membrane density. 10
2. Changes in deuterated to protonated fatty acid ratio at the ICM and whole cell level during asynchronous and synchronous growth 13
3. The effect of deuterium labeling on phospholipid acyl group turnover 14

IV. DISCUSSION 24

V. REFERENCES 27

VI. APPENDIX A 28

VII. CURRICULUM VITAE 30

LIST OF FIGURES

Figure | Page

1. Mass spectrometric analysis of deuterated and non-deuterated methyl-vaccenate 12
2. Changes in the deuterated to protonated vaccenic acid ratio in asynchronous cultures, whole cell and ICM. . . 16
3. Changes in the deuterated to protonated vaccenic acid ratio in synchronous cultures, whole cell and ICM . . . 18
4. Acyl group turnover in a non-deuterated culture 21
5. Acyl group turnover in a culture shifted from D_2O to H_2O based medium. 23

Introduction

Rhodopseudomonas sphaeroides, a Gram-negative nonsulfur purple photosynthetic bacterium, has been used extensively for investigations of the mode and regulation of membrane biogenesis (15, 9). When grown chemotrophically (aerobic-dark), *R. sphaeroides* displays a respiratory metabolism and only possesses the cytoplasmic and outer membrane systems characteristic of its Gram-negative phenotype. In contrast, phototrophically (anaerobic-light) grown cells possess an extensive intracytoplasmic membrane (ICM system) that occurs in conjunction with the membranes comprising their Gram-negative envelope. Although physically contiguous with the cytoplasmic membrane, the ICM is compositionally distinct and is known to harbor the primary and auxilliary photopigments required for photosynthetic growth.

Numerous studies have been conducted on the structure, function and biogenesis of the ICM of *R. sphaeroides* (for review see ref. 4). Upon disruption of phototrophically grown cells, a comminution of the ICM occurs resulting in the generation of a population of uniform unilamellar vesicles. These ICM vesicles are easily obtained in purified form by either gel-filtration chromatography, or by

---

The format followed in this text is that of the Journal of Bacteriology, a publication of the American Society for Microbiology.

passage through a discontinuous sucrose gradient, and their composition has been well defined chemically. Purified ICM vesicles display a protein-to-phospholipid ratio of approximately 3 and possesses over 40 different proteins. Six to eight of these proteins are specifically associated with the primary photopigments and collectively comprise greater than 80% of the total ICM protein. The ICM phospholipid and fatty acid profiles are also quite distinctive. As is typically observed in bacterial systems, phosphatidylethanolamine is the dominant phospholipid (43-46%) and phosphatidylglycerol (33-36%) and cardiolipin (2-3%) are also easily demonstrable. Interestingly, however, ICM vesicles also possess significant quantities of phosphatidylcholine (15-20%); a phospholipid not normally associated with procaryotes. The fatty acid composition of the ICM phospholipid fraction consists of over 90% cis-vaccenic acid ($C_{18:1}^{\Delta 11}$).

Recently, information concerning the mode and regulation of ICM assembly has been greatly augmented by studies employing synchronously dividing cell populations of *R. sphaeroides* (1, 5, 9). By utilizing deuterium oxide as a nonspecific ICM density label, Lueking *et al.* (5) showed that the ICM from cells synchronized in D_2O-based medium undergoes discontinuous decreases in density when the cells are allowed to divide synchronously in H_2O-based medium. Since these observed abrupt decreases in ICM specific density occurred just prior to cell division, these results suggested that a "light" component(s) synthesized during growth in the H_2O-based medium was being produced and/or inserted into the heavy "ICM" in

a cell cycle specific manner. This proposal was later confirmed by investigations of the kinetics of appearance of ICM specific components. These studies showed that only the ICM phospholipid components were synthesized, or inserted, in a discontinuous fashion. ICM associated proteins and photopigments were found to be continuously produced and inserted into the ICM of cells undergoing synchronous growth. Thus, although the quantitative distribution of ICM associated deuterium was not examined, it was proposed that the abrupt decreases in ICM specific density were due to the dilution of preexisting "heavy" ICM phospholipids with newly synthesized phospholipids.

The precise nature of the relationship between ICM biogenesis and the biogenesis of the cytoplasmic and outer membranes of *R. sphaeroides* is unknown. Interestingly, by employing a double isotopic labeling procedure, Fraley *et al.* (9) reported that the pattern of phospholipid synthesis observed at the whole cell level does not directly reflect the pattern of phospholipid production (or insertion) of those phospholipids associated with the ICM. This latter observation suggests that the production of phospholipids associated with the three membrane systems possessed by *R. sphaeroides* may be differentially controlled.

The present study provides quantitative information on the extent of deuteration of ICM phospholipid acyl moieties and, further, utilizes deuterium labeling of acyl moieties to monitor the differential formation of whole cell and ICM associated phospholipids. The

results obtained have been utilized to develop a proposal concerning the biogenesis of the ICM of *R. sphaeroides*.

MATERIALS AND METHODS

Bacterial strains, media, and conditions of growth. *Rhodopseudomonas sphaeroides* strain M29-5 (Met^-, Leu^-), derived from strain 2.4.7, was obtained from Samuel Kaplan, University of Illinois, Urbana. This organism was grown in a succinic acid minimal medium supplemented with 40 μg per ml of both L-methionine and L-leucine (4). The non-specific deuterium labeling of cells was accomplished by allowing the culture to undergo eight to ten mass doublings in this same minimal medium adjusted to 80% deuterium oxide. Stock cultures of normal and deuterated cells were maintained at -20°C in the appropriate medium adjusted to 10% (wt./vol.) glycerol.

For studies involving the determination of acyl group turnover, cells were adapted and grown in succinic acid minimal medium that was additionally supplemented with 2% (wt./vol.) casamino acids and 40 μg per ml of acetic acid added in the form of sodium acetate. ^{14}C-acetic acid (New England Nuclear) was used at 0.1 μC/ml.

All incubations were conducted anaerobically in the light (photoheterotrophically) in either completely filled screw-capped tubes or in flat-walled vessels (1 - 5 liters) under an atmosphere of 95% N_2, 5% CO_2. Incubations were conducted 32°C with saturating illumination (500 ft-c) provided by a blank of Lumiline lamps (Sylvania). The inoculum source for all studies consisted of cells previously adapted to logarithmic phase growth in the appropriate medium. Culture growth was monitored turbidimetrically using a Klett-Summerson colorimeter equipped with a no. 66 filter. A culture turbidity

of 100 photometer units corresponds to a value of 270 μg of cellular dry weight per ml and a cell density of 10^9 cells per ml.

Culture synchronization procedure. The generation of division synchrony in phototrophically growing cultures of *R. sphaeroides* was accomplished employing the light-cycling procedure described by Lueking *et al*. (13). Asynchronously dividing cell populations adapted to growth at 500 ft-c were immediately shifted to a light intensity of 50 ft-c and incubated at this light intensity for 3.5 hours. Upon reincubation at 500 ft-c, these cultures displayed a high degree of division synchrony.

Cell enumeration. The presence of division synchrony was determined by monitoring total cell number employing a Petroff-Hausser counting chamber. Culture samples (1 ml) were removed at 15 minute intervals and transferred to a tube containing 1 ml of 5% (wt./vol.) formaldehyde. All determinations involved the counting of 500 to 900 cells.

Intracytoplasmic membrane purification. ICM preparations utilized for the determination of the extent of ICM associated fatty acid deuteration were purified as described by Fraley *et al* (9). Culture samples adjusted for uniform cell mass were removed at timed intervals and the cells harvested for centrifugation at 12,000 x g for 10 minutes. The pelleted cells were then washed once with 0.1 M sodium phosphate buffer (pH 7.6) containing 0.01 M ethylenediaminetetracetic acid (EDTA) and 0.05 M β-mercaptoethanol. The washed cell pellet was then resuspended in 4 ml of this buffer and the cells were

disrupted by sonication for 10 minutes (35% efficiency; 40% duty cycle) with a Branson Model W-350 sonicator. Following treatment with DNase and RNase (5 μg/ml, each) the crude extract was centrifuged at 12,000 x g for 10 minutes to remove unbroken cells and cellular debris. The supernatant obtained was centrifuged at 106,000 x g for 1 hour and the resulting crude ICM particulate fraction was resuspended in 0.3 ml of phosphate buffer containing 7% (wt./vol.) sucrose and was layered onto a 1 x 25 cm column of Sepharose 2B previously equilibrated with phosphate buffer, pH 7.6. The column was eluted with this same buffer (15 ml/hr) and the collected fractions (1 ml) were monitored for their absorbance at 280 nm (protein) and 850 nm (bacteriochlorophyll). Those fractions exhibiting a constant OD_{280}/OD_{850} ratio, representing pure ICM vesicles, were pooled and the purified vesicles were collected by centrifugation at 106,000 x g for 1 hour. Purified ICM vesicles were resuspended in a minimal volume of phosphate buffer and stored at 4°C.

Phospholipid extraction. The phospholipids present in whole cells and purified ICM preparations were extracted by the method of Bligh and Dyer (7) as described by Ames (8). Phase partitioning and washing of the chloroform extracts were conducted as described by Lueking *et al.* (13). The neutral lipids and photopigments contained in the washed chloroform extracts were removed by chromatography of the samples on columns of silicic acid. Samples were applied to columns of silicic acid slurried and equilibrated in chloroform methanol (49/1, vol./vol.) and neutral lipids and photopigments were

eluted with 10 column volumes of this same solvent system. The column was then eluted with 10 column volumes methanol to remove the polar lipid fraction containing the cellular phospholipids.

Preparation of fatty acid methyl esters. Phospholipid acyl moieties were directly converted to their corresponding fatty acid methyl esters by acid catalyzed transesterification. Polar lipid fractions obtained by silicic acid chromatography were taken to dryness under an atmosphere of nitrogen and were redissolved in 2 ml of anhydrous, methanolic-HCl. Acidified methanol was prepared by the addition of 5.0 ml acetyl chloride to 50 ml of cool, anhydrous methanol. Transesterifications were conducted in screw-capped tubes at 70°C for 3 hours. Following the period of transesterification, 5 ml of 5% (wt./vol.) sodium chloride was added to the transesterification mixture and the fatty acid methyl esters were extracted by washing the mixture three times with 3 ml volumes of hexane. The hexane fractions were then collected and washed with 4 ml of 2% (wt./vol.) potassium bicarbonate to remove residual free fatty acid and the final, washed hexane fraction was then dried by storage over sodium sulfate.

Purification of fatty acid methyl esters. Monounsaturated fatty acid methyl esters (cis-vaccenate methyl esters) were separated from saturated fatty acid methyl esters by chromatography on thin layer plates of silica gel G impregnated with 10% (wt./wt.) silver nitrate. Thin layer plates were developed in a solvent system composed of hexane/diethyl ether (9:1, vol./vol.). Samples were visualized by

spraying the plates with a solution of 0.1% (wt./vol.) 2,7-dichlorofluoroscien and the areas of the plate corresponding to the unsaturated fatty acid methyl esters were removed and the methyl esters eluted from the silica gel with 3 ml of ethyl acetate.

Gas chromatographic resolution of deuterated and protonated species of cis-vaccenyl methyl esters. Deuterated and protonated species of methyl vaccenate were separated by wall-coated open tubular (WCOT) column gas chromatography on a Tracor 660 gas-chromatograph equipped with a 50 meter capillary column coated with methyl silicone (SE-30). Separations were conducted isothermally at a column temperature of 220°C, injection port temperature of 250°C and a detector (FID) temperature of 280°C. Carrier-gas (He) flow rate was adjusted to 3 ml/min. The quantitation of methyl vaccenate was accomplished by peak area integration employing the Numonics model 1224 electronic digitizer programmed for area closure.

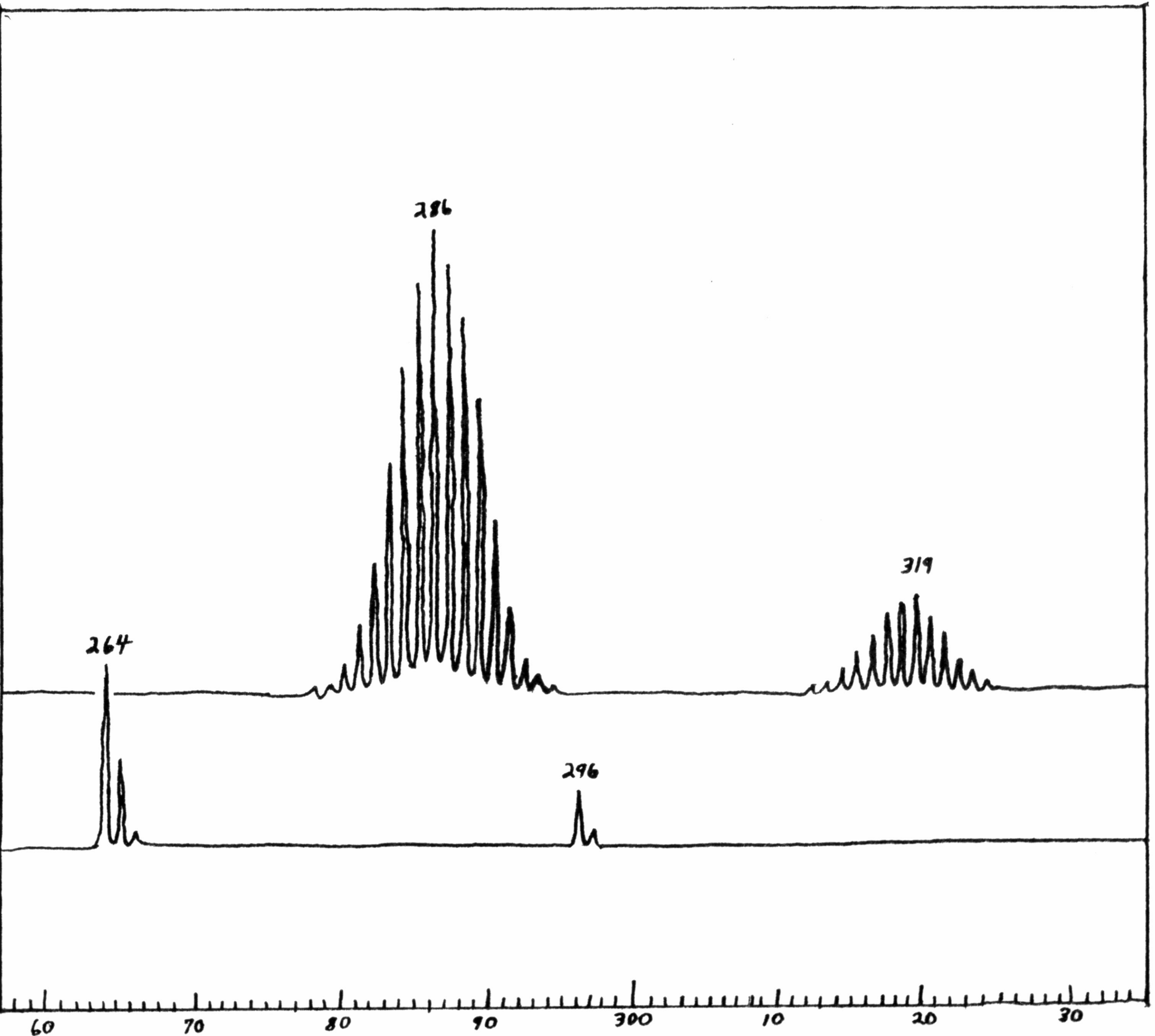
Mass spectrometric analysis of deuterated methyl vaccenate. The extent of deuteration of methyl vaccenate prepared from cells grown in D_2O (80%) based medium was determined directly by mass spectrometry. Mass spectra were obtained using an LKB-9000 GC-Mass Spectrometer. The ion source was maintained at 290°C. Accelerating voltage was 3.5 kV and electron energy was 70 eV. Material emerging from a 50 m capillary column packed with OV-1 (180°C) were repeatedly scanned to obtain mass spectra.

RESULTS

The increased molecular weight of deuterated vaccenic acid and its contribution to increased membrane density. Previous studies have shown the specific density of non-deuterated ICM to be 1.18 g/cm^3. This compares to a specific density of 1.22 g/cm^3 for deuterated ICM purified from cells grown in 80% D_2O based medium. The 3.38% increase in density results from the contribution of the increased densities of all deuterated components. How much each component contributes to ICM density has not been previously investigated. For this reason, we decided to pursue an investigation to determine the contribution made by the deuteration of the acyl moieties of the phospholipids. This decision was based on the evidence that the discontinuous synthesis or insertion of phospholipids into the ICM of a synchronously dividing cell population, which has undergone a density shift, produced a marked discontinuous decrease in ICM density. For this to occur, the contribution of membrane phospholipid to increased density of deuterated ICM must be significant.

To initiate our studies, the predominant fatty acid (*cis*-vaccenic acid) was isolated from cells of *R. sphaeroides* grown in 80% D_2O medium as described in Materials and Methods. Mass spectrometric analysis showed an average increase of 23 mass units (23 deuterium atoms per molecule) per fatty acid molecule (Fig. 1). This corresponded to a 6% increase in the molecular weight of an

Fig. 1. Mass spectra of deuterated and protonated purified methyl vaccenate: Lower line shows protonated species. Upper line shows deuterated species. Mass spectra have been abbreviated for clarity.



286
319
264
296
60
70
80
90
300
10
20
30

individual phospholipid molecule. Equating the 6% increase in molecular weight to a 6% increase in density, its was possible to calculate the expected density of ICM deuterated only at the acyl groups of the phospholipids. The calculated value, (using the 70 wt.% protein and 30 wt.% phospholipid calculated to be present in non-deuterated ICM), was 1.208 g/cm^3. This was a 2.377% increase over the density of non-deuterated ICM and represented approximately 70% of the observed increase in density of deuterated ICM. (See calculations, Appendix A).

Changes in deuterated/protonated fatty acid ratio at the ICM and whole cell level during asynchronous and synchronous growth. Fraley *et al* (9) reported that phospholipid synthesis at the whole cell level does not reflect the pattern of synthesis and/or insertion of ICM associated phospholipids. To investigate this report, the non-specific ICM deuterium labeling technique of Lueking *et al*. (5) was utilized and changes in the deuterated/protonated fatty acid ratio was monitored at the ICM and whole cell level.

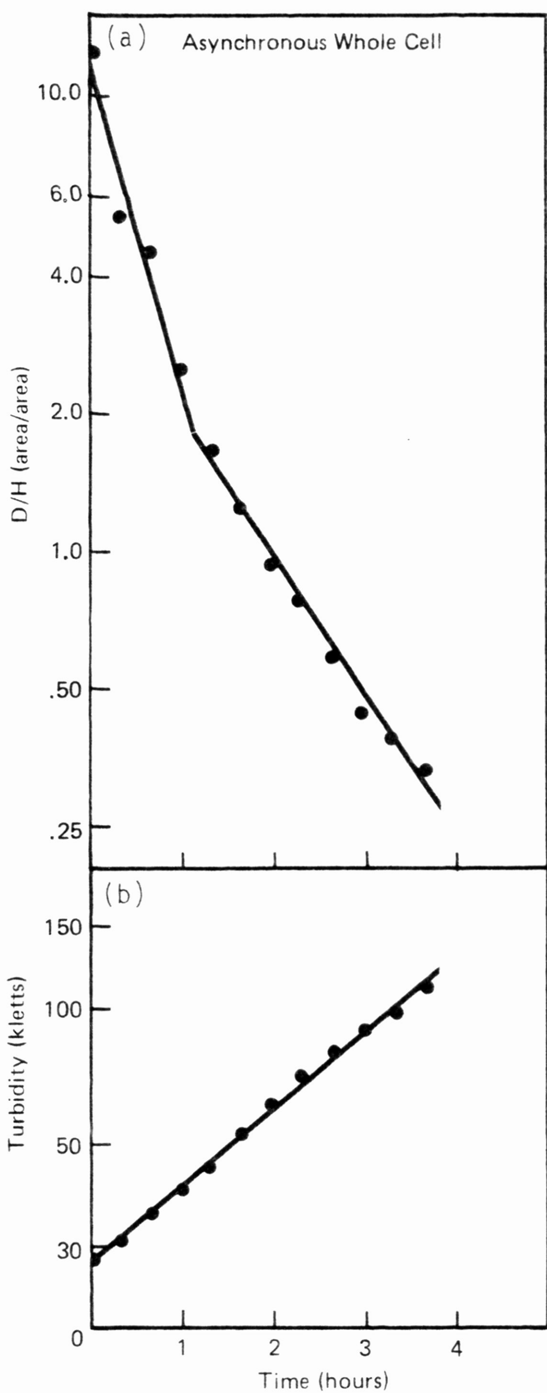
Cultures of *R. sphaeroides* grown in 80% D_2O-based medium (8 to 10 mass doublings) were used to inoculate H_2O-based medium and the cells were grown asynchronously. Samples adjusted for uniform cell mass were removed at timed intervals and the phospholipids extracted and the methyl-vaccenate methyl esters isolated as described in Materials and Methods. Separation of the deuterated and protonated species of methyl-vaccenate was accomplished by gas chromatography. This was followed by measurement of peak areas for each sample and calculation of the deuterated/protonated (D/H) ratios.

The observed changes in the D/H ratio at the whole cell level (Fig. 2a) showed that dilution of preexisting whole cell vaccenic acid occurred approximately as a function of cell growth (Fig. 2b), although at a slightly faster rate than expected. The same results were obtained with ICM associated vaccenic acid. The D/H ratio again declined approximately as a function of cell growth (Figs. 2c and 2d). Changes in the D/H ratio were also followed in synchronous cell populations.

The light-cycling technique by Lueking _et al_. (13) was employed to establish synchronously dividing cultures in H_2O-based medium. Deuterium labeling was accomplished as described earlier. Examination of the D/H ratio of vaccenic acid at the whole cell level during synchronous growth showed that dilution of preexisting deuterated methyl vaccenate occurred as a function of cell growth, although again slightly faster than expected (Fig. 3a). However, the decline in the D/H ratio did not reflect the cyclic increase in cell number (Fig. 3b). Concomitant with the observed discontinuous increase in total cell number (Fig. 3d), the D/H ratio of ICM vaccenic acid demonstrated a stepwise decline (Fig. 3c). The rapid decline in the ratio occurring just prior to cell division; a result suggestive of the discontinuous decrease in specific density of ICM described by Lueking _et al_ (5).

The effect of deuterium labeling on acyl group turnover. The results previously presented suggested that increased phospholipid

Fig. 2. Changes in the deuterated to protonated vaccenic acid ratio in asynchronous cultures: (a) Deuterated to protonated ratio whole cell. (b) Culture turbidity. (c) Deuterated to protonated ratio of purified ICM. (d) Culture turbidity.


(a)
Asynchronous Whole Cell
10.0
6.0
4.0
2.0
1.0
.50
.25
D/H (area/area)
(b)
150
100
50
30
0
Turbidity (kletts)
1
2
3
4
Time (hours)

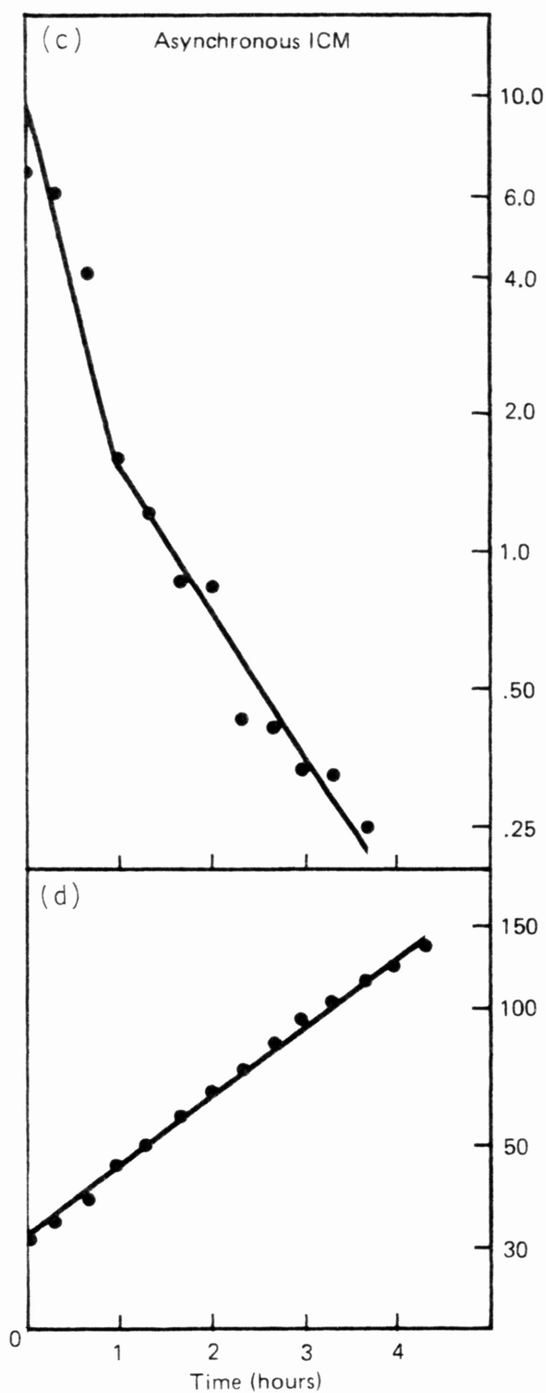
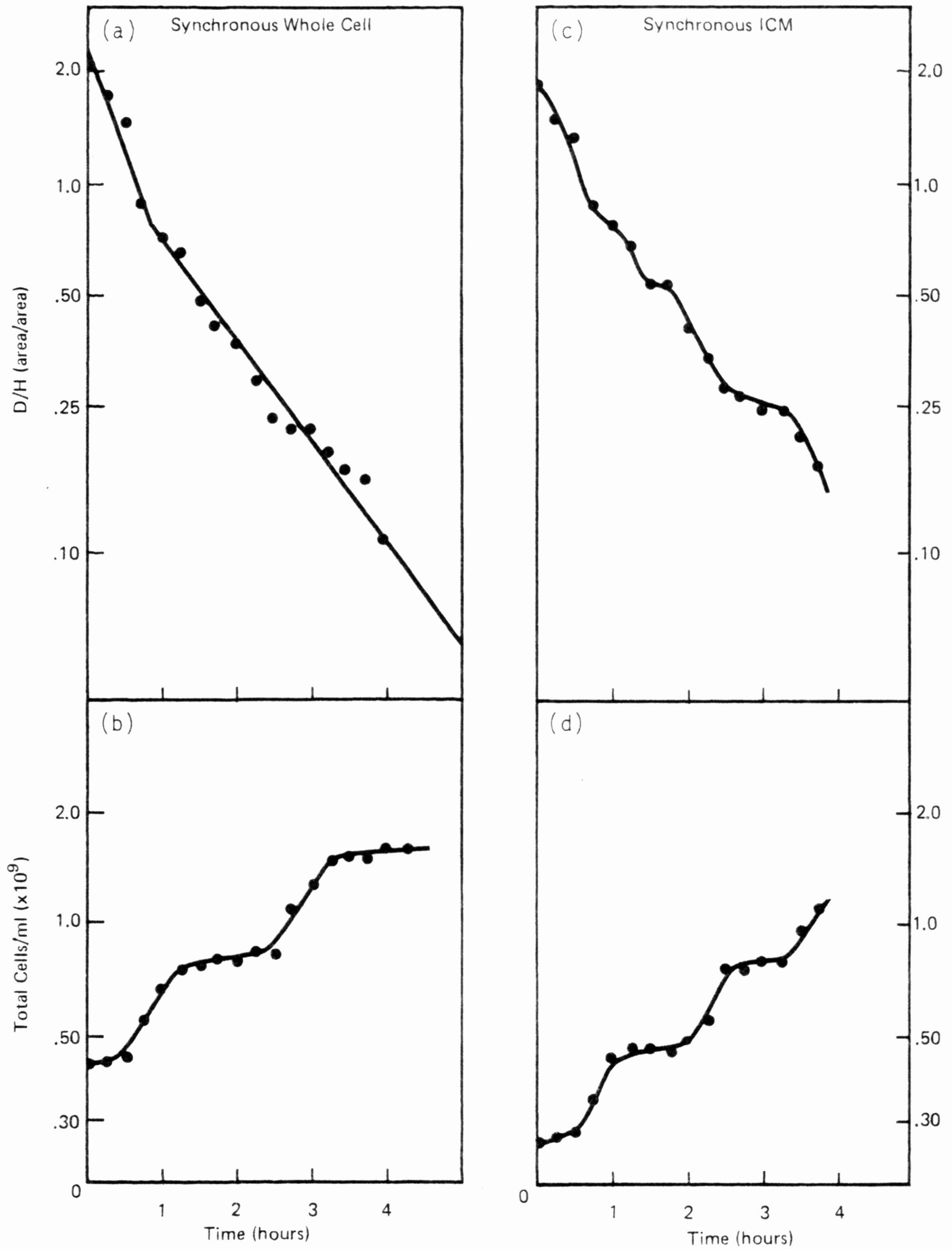

(c)
Asynchronous ICM
10.0
6.0
4.0
2.0
1.0
.50
.25
(d)
150
100
50
30
0
1
2
3
4
Time (hours)

Fig. 3. Changes in the deuterated to protonated vaccenic acid ratio in synchronous cultures: (a) Deuterated to protonated ratio whole cell. (b) Total cells per ml of culture. (c) Deuterated to protonated ratio purified ICM. (d) Total cells per ml of culture.



(a)
Synchronous Whole Cell
(c)
Synchronous ICM
D/H (area/area)
2.0
1.0
.50
.25
.10
(b)
(d)
Total Cells/ml (x10^9)
2.0
1.0
.50
.30
0
1
2
3
4
Time (hours)

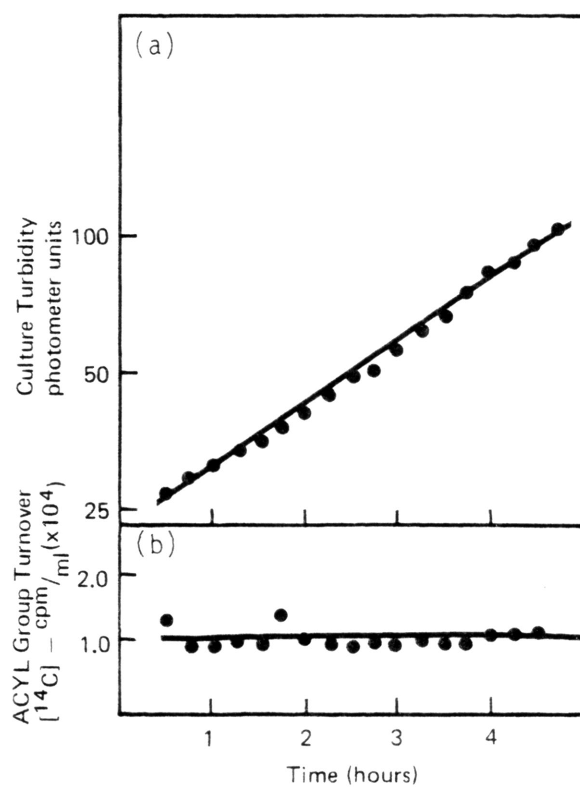
synthesis and/or acyl group turnover might be responsible for the increased rate of dilution of deuterated vaccenic acid. Investigations as to the effect of deuterium labeling on acyl group turnover were performed on a non-deuterated asynchronous culture and an asynchronous culture shifted from D_2O to H_2O-based medium.

The non-deuterated culture was allowed to undergo 8 to 10 mass doublings in acetate medium containing .1 μCi/ml ^{14}C-acetic acid. The cells were then washed and placed in the cold acetate medium. Samples of uniform volume were removed at timed intervals, the phospholipids extracted, and ^{14}C- cpm per ml of culture determined. The data showed that no decrease in the original radioactivity occurred (Fig. 4b).

The same study was performed on a culture grown in 80% D_2O-based acetate medium containing .1 μCi/ml ^{14}C-acetic acid. The cells were washed free of D_2O and ^{14}C- acetic acid prior to inoculation of cold, non-deuterated medium. Uniform samples were removed at timed intervals and ^{14}C- cpm per ml determined. The results indicated a significant decrease in radioactivity of the phospholipid extracts over the course of the experiment (Fig. 5b). This suggested that the acyl groups were being turned over, following a density shift.

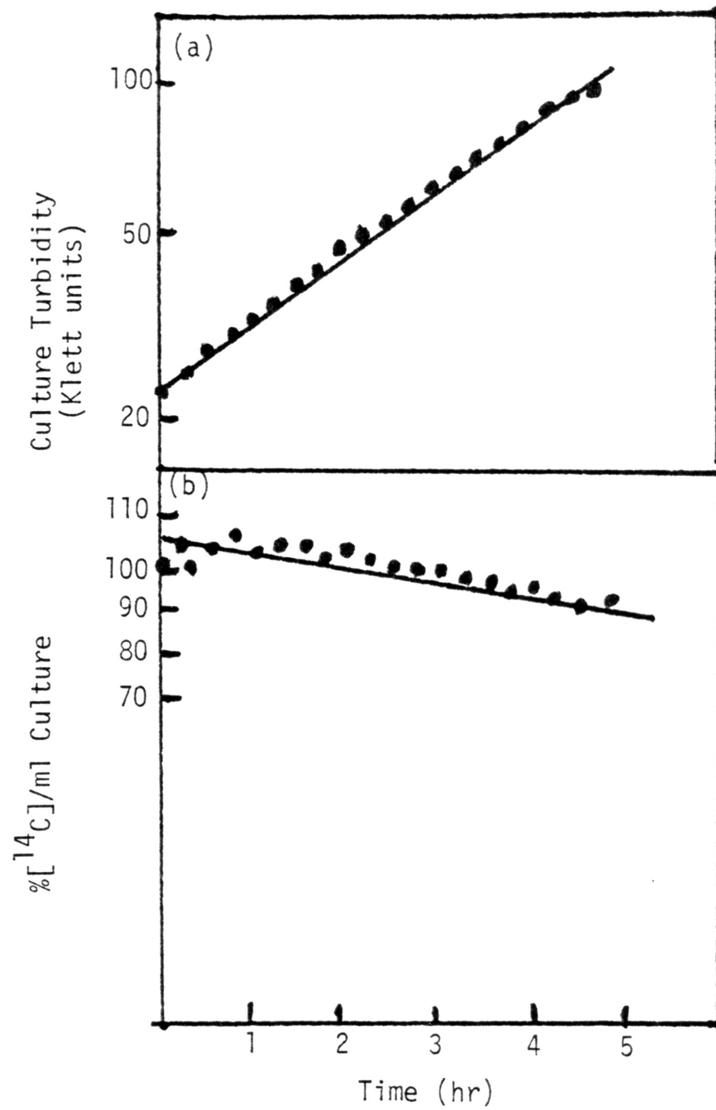
At this time, no investigations as to the rate of phospholipid synthesis have been performed on cultures undergoing a density shift.

Fig. 4. Acyl group turnover in a non-deuterated culture: (a) Culture turbidity. (b) Radioactivity per ml of culture.



(a)
Culture Turbidity
photometer units
100
50
25
(b)
ACYL Group Turnover
[14C] – cpm/ml (x10⁴)
2.0
1.0
1
2
3
4
Time (hours)

Fig. 5. Acyl group turnover in a culture shifted from D_2O to H_2O-based medium: (a) Culture turbidity. (b) Radioactivity per ml of culture.


(a)
100
50
20
Culture Turbidity
(Klett units)
(b)
110
100
90
80
70
%[^{14}C]/ml Culture
1
2
3
4
5
Time (hr)

DISCUSSION

Extensive investigations of the differential synthesis and/or insertion of ICM phospholipids in cells of *R. sphaeroides* have been conducted. Initial studies by Lueking *et al*. (13) (Introduction), demonstrated that ICM specific density undergoes discontinuous decreases in a synchronously dividing culture shifted from D_2O to H_2O-based medium. However, no investigations were conducted to quantitate the extent of deuteration of the membrane component's; in particular, the membrane phospholipids.

The results of the present study showed that the extent of acyl group deuteration of the ICM phospholipid fraction could account for approximately 70% of increased density of the ICM derived from cells grown in 80% D_2O medium. This is especially surprising, since phospholipid composes only 30 wt. % of the composition of normal ICM. Whether the wt. % of ICM phospholipid is increased in a deuterated membrane is not presently known. Also of interest is the close agreement of our results concerning deuteration of the acyl groups with results obtained from eukaryotic systems. Wadke *et al*. (10) has shown that 22 deuterium atoms are incorporated per acyl group when rat liver is perfused with 80% D_2O. The close agreement of Wadke's results (10) with our findings suggests that our calculations are accurate.

The results obtained from the studies which monitored the change in the D/H ratio of methyl vaccenate in synchronous and asynchronous

cell populations suggest that synthesis of phospholipids at the whole cell level is a continuous process associated with the cytoplasmic membrane, and that the CM produced phospholipid is then discontinuously inserted into the ICM. If phospholipid synthesis was occurring in both membrane systems, one would expect the whole cell D/H ratio of a synchronous culture to decrease discontinuously due to the cummulative addition of continuously synthesized cytoplasmic membrane phospholipids to discontinuously synthesized ICM phospholipids. This interpretation is possible based on previous studies which proposed that the ICM is continuous with the cytoplasmic membrane (11). Also, a specific phospholipid transport protein(s) has been demonstrated in cell free extracts of R. sphaeroides (12), which would provide a molecular basis for the movement of phospholipids from the cytoplasmic membrane to the ICM.

The biphasic nature of the graphs of the D/H ratio of methyl vaccenate versus time indicate that dilution of the preexisting "heavy" components is occurring faster than cell growth. The second portion of the biphasic decrease, which is still declining faster than cell growth, might be explained by acyl group turnover observed in cells undergoing a density shift. However, the initial rapid decrease is too great to be explained by acyl group turnover. This is probably due to increased phospholipid synthesis and will be investigated as soon as possible. A previous study by Fraley et al. (9), using ^{32}P-labeling of cellular phospholipids showed that no

significant phospholipid turnover occurred in synchronously dividing cell populations.

The present investigation has clearly demonstrated the sensitivity of acyl group deuterium labeling as a technique to monitor fatty acid production via changes in the deuterated to protonated fatty acid ratio. This technique is more sensitive than earlier techniques which monitored changes in the specific density of the ICM and also, has the added advantages of allowing one to monitor changes in the D/H ratio at the level of the whole cell. Additionally, it is readily reproduced as evidence by the repeated experiments. We feel that this technique will be of great utility in investigations of the temporal regulation of membrane assembly in other systems as well as continued investigations of ICM assembly in *R. sphaeroides*.

REFERENCES

1. Fraley, T., Lueking, D. 1977. J. Biological Chem. 253, 458-464.

2. Fraker, P., Kaplan, S. 1971. J. Bacteriol. 108, 465-473.

3. Kaplan, S., Fraley, R. T., and Lueking, D. R. 1979. In: Microbiology 1979. Schessinger, D., Ed. American Society for Microbiology. 26-29.

4. Kenyon, C. N. 1978. In: The Photosynthetic Bacteria, Clayton, R. K. and Sistrom, .W R., Eds. 281-310.

5. Lueking, D. R., Fraley, R. T., and Kaplan, S. 1978. J. Biological Chem. 253, 451-457.

6. Cohen-Bazise, G., Sistrom, W. R., and Stanier, R. Y. 1957. J. Cell. Comp. Physiol. 49, 25-68.

7. Bligh, E. G., and Dyer, W. J. 1959. Can. J. Biochem. Physiol. 37, 911-917.

8. Ames, G. F. 1968. J. Bacteriol. 95, 833-843.

9. Fraley, T., Lueking, D. R., Kaplan, S. 1979. J. Biological Chem. 254, 1980-1986.

10. Wadke, M., Brunengraber, H., Lowenstein, J. M,. Dolhun, J. J., and Arsenault, G. P. 1977. Biochemistry. 12, 2619-2624.

11. Oclzc, J. and Drews, G. 1972. Biochim. Biophys. Acta 265, 209-239.

12. Cohen, L. K., Lueking, D. R., and Kaplan, S. 1979. J. Biological Chem. 254, 271-278.

13. Lueking, D. R., Campbell, T. B., Burghardt, R. C. 1981. J. Bacteriol. 146, 000-000.

Appendix A

There are 23 deuterium atoms per acyl group. Two acyl groups per phospholipid means an increase in molecular weight of 46.

Avg. M. W. Phospholipid = 775

% increase M. W. $= \frac{46}{775} \times 100 = 6\%$

Density Phospholipid $= .92\ g/cm^3$

Increased Density $= (.92)(.06) + .92$

$= .975\ g/cm^3$

$\bar{v}$ (partial specific vol.) $= \frac{1}{\text{density}}$

$= \frac{1}{.975}$

$= 1.025\ cm^3/g$

Calculation of wt. % of protein and phospholipid present in non-deuterated ICM; density $1.18\ g/cm^3$.

Equation 1: $\bar{v}_{ICM} = \bar{v}_{Protein}(\text{wt.\% protein}) + \bar{v}_{p\text{-}lipid}(\text{wt.\% P-lipid})$

$\bar{v}_{\text{Non-deut. ICM}} = \frac{1}{1.18}\ g/cm^3 = .847\ cm^3/g$

$\bar{v}_{Protein} = \frac{1}{1.34}\ g/cm^3 = .74\ cm^3/g$

$\bar{v}_{Phospholipid} = \frac{1}{.92}\ g/cm^3 = 1.09\ cm^3/g$

Substituting into Equation 1:

$$.847 = .74(x) + 1.09\ (1-X)$$
$$.847 = .74X + 1.09 - 1.09X$$
$$1.09X - .74X = 1.09 = .847$$
$$.34X = .243$$
$$X = .694 \text{ or } 69.4 \text{ wt.\% protein}$$
$$1 - .694 = .306 \text{ or } 30.6 \text{ wt.\% phospholipid}$$

Using 70 wt. % protein and 30 wt. % phospholipid, the density of a membrane deuterated only at the acyl groups may be calculated as follows:

$$\bar{v}_{deut.\ ICM} = \bar{v}_{protein}\,(.694) + \bar{v}_{deut.\ p\text{-}lipid}\,(.306)$$

$$\bar{v}_{deut.\ ICM} = .74\ (.694) + 1.025(.036)$$

$$\bar{v}_{deut.\ ICM} = .514 + .314$$

$$\bar{v}_{deut.\ ICM} = .828\ cm^3/g$$

$$\text{Density} = \frac{1}{.828}\ cm^3/g = 1.208\ g/cm^3$$

$$\%\ \text{theoretical increase} = \frac{1.208\ g/cm^3 - 1.18\ g/cm^3 \times 100\%}{1.18\ g/cm^3}$$

$$= 2.37\%$$

$$\%\ \text{observed increase} = \frac{1.22\ g/cm^3 - 1.18\ g/cm^3 \times 100\%}{1.18\ g/cm^3}$$

$$= 3.38\%$$

Approximate % increase attributable to deuteration of acyl groups:

$$\%\ \text{increase} = \frac{.237 \times 100\%}{.338}$$

$$= 70.1\%$$