Phospholipid Composition of *Pneumocystis carinii carinii* and Effects of Methylprednisolone Immunosuppression on Rat Lung Lipids

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The phospholipid class composition of *Pneumocystis carinii carinii* freshly isolated from infected lungs generally resembled that of the host lung, suggesting that the parasite scavenges lung alveolar lipids. However, subtle quantitative differences were demonstrated, indicating that the pathogen has the metabolic capacity to de novo synthesize, or at least tailor, its lipids. The concentration of phosphatidylcholine, the major lung surfactant lipid, in the organism was lower than that in lungs of normal and immunosuppressed uninfected rats, and the concentration of phosphatidylinositol was higher. Phosphonolipids were not detected in the organism by chemical analysis and nuclear magnetic resonance spectrometry. The immunosuppressive regimen alone caused increases in both surfactant protein A and the lipid content of the whole lung. The lungs of rats that were subjected to corticosteroid immunosuppression and had heavy parasite loads had dramatically elevated surfactant protein A levels, whereas the lipid contents of these lungs were not different from lipid contents in whole lungs of immunosuppressed uninfected rats. *P. carinii* was found to concentrate lipids, indicating that a large amount of the lipids in the whole infected rat lung was within the parasites residing in the organ. These observations have important implications relevant to the use of corticosteroid therapy for *P. carinii* pneumonitis.

Pneumocystis carinii resides in the lung alveolus bathed in pulmonary surfactant lipids and proteins secreted by epithelial type II cells. Pulmonary surfactant is composed of 90% lipids with lesser amounts of carbohydrates and proteins. The composition of lung surfactant lipids is distinct. Phosphatidylcholine (PC) constitutes about 80% of surfactant lipids, and more than half of the PC molecules contain disaturated (dipalmitoyl) fatty acids. Phosphatidylglycerol accounts for 10%, and phosphatidylinositol (PI), cholesterol, and other lipids together account for the remaining 10% of surfactant lipids (9, 23).

In 1983, Kernbaum et al. (16) reported that bronchoalveolar lavage fluid (BALF) of *P. carinii*-infected rats had reduced amounts of phospholipids compared with BALF from normal rats or cortisone-treated controls. They also measured elevated levels of phospholipase activity in BALF of *P. carinii*-infected lungs, which, they concluded, accounted for the diminished levels of BALF surfactant phospholipids. Subsequently, Sheehan et al. (23) confirmed the dramatic decrease in surfactant phospholipids in BALF from *P. carinii*-infected lungs, but they reported that corticosteroid (dexamethasone) treatment itself, in the absence of *P. carinii* infection, caused an increase in BALF phospholipase A₂ activity. They suggested that the decrease in surfactant lipids might be the result of a parasite-specific effect on the synthesis or secretion of surfactant by pneumocytes. Since those earlier studies, an apparent paradox has been observed by several workers. While there is decreased surfactant lipid, surfactant protein (surfactant protein A [SP-A] and SP-D) levels are increased in BALF of *P. carinii*-infected lungs (17, 27).

Several studies indicate that *P. carinii* effectively scavenges exogenously available lipids. It can take up complex polar lipids such as intact phospholipids (24). Thus, it is likely that

the bulk of phospholipids present in *P. carinii* are taken up as intact molecules and that the phospholipid composition of the organism would strongly reflect the composition of the lung surfactant. The phospholipid composition of *P. carinii* was examined in an earlier study on preparations that very likely contained substantial host contaminant molecules (19). Recently, a highly pure organism preparation which was found to be suitable for lipid analysis was developed and characterized (14). These preparations were used in the present study for detailed analyses of the phospholipid content and the relative amounts of the major classes present in the organism's lipids. These values were compared with those of lipids extracted from whole lungs of (i) normal, (ii) immunosuppressed uninfected, and (iii) *P. carinii*-infected rats. The lipids were also analyzed for phosphonolipids.

MATERIALS AND METHODS

Rat lungs and organism preparations. *Pneumocystis carinii carinii* (20) organisms were isolated from female Lewis rats and purified by procedures reported earlier (14). Briefly, the rats were immunosuppressed by subcutaneous injections of methylprednisolone acetate (Depo-Medrol; Upjohn Co., Kalamazoo, Mich.) and infected by intratracheal intubation of previously frozen *P. carinii* preparations that had been tested negative for ubiquitous bacteria and fungi (4, 14). The organisms were isolated by a protocol that included perfusion (to eliminate blood elements), mincing and sieving of the excised lungs in the presence of the mucolytic agent glutathione, and homogenization in a Stomacher apparatus (Tekmar, Cincinnati, Ohio). Purification involved washings by a series of high-speed and low-speed centrifugation steps to eliminate the lung surfactant and cell debris, followed by membrane filtration. The purity of this preparation was fully characterized by light and electron microscopy, quantitation of total protein in process control samples, and the elimination of SP-A. Furthermore, the elimination of exogenously added sterol and the phospholipid profile of the final preparation documented the purity of the preparation (14).

Whole lungs from normal rats and from rats immunosuppressed by corticosteroid treatment (but uninfected) served as controls. In this study, whole lungs heavily infected with *P. carinii* were also analyzed. Excised lungs were minced and then disrupted in a French pressure cell (Aminco, Urbana, Ill.) at 20,000 lb/in².

Extraction and fractionation of lipids. Total lipids were extracted by the method of Bligh and Dyer (3) and purified by using the method of Folch et al. (8); then, the neutral and polar lipid fractions were separated by adsorption column chromatography (Unisil; Clarkson Chemical Co., Williamsport, Pa.)

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TABLE 1. Lipid and SP-A contents of purified preparations of rat-derived *P. carinii carinii* and of whole lungs from control and infected rats^a

Sample	Total lipids		Polar lipids (mg/ mg of protein) ^b	Phospholipids ^c		Neutral lipids/polar lipids ^d	SP-A (μg/mg of protein)
	mg/mg of protein	mg/g (wet wt)		μmol of P/mg of protein	μmol of P/g (wet wt)		
Lungs							
Normal	0.24 ± 0.03 (6)	19.2 ± 2.1 (6)	0.2 ± 0.0 (6)	0.16 ± 0.01 (6)	13.3 ± 2.0 (6)	0.57 ± 0.03 (22)	1.1 ± 0.2 (5)
Immunosuppressed uninfected	0.34 ± 0.06 (5)	27.3 ± 7.5 (5)	0.2 ± 0.0 (4)	0.26 ± 0.04 (4)	18.5 ± 5.7 (5)	0.61 ± 0.08 (17)	2.0, 3.7 (2)
<i>P. carinii</i> infected ^e	0.32 ± 0.02 (4)	28.1 ± 2.4 (4)	0.3 ± 0.0 (4)	0.20 ± 0.01 (4)	18.1 ± 1.2 (4)	0.52 ± 0.03 (4)	37.6, 13.1 (2)
<i>P. carinii</i>	0.9 ± 0.2 (5)	26.7 ± 3.5 (3)	0.52 ± 0.08 (4)	0.37 ± 0.03 (8)	11.1 ± 1.2 (3)	0.60 ± 0.05 (18)	<0.5 ^f

^a The data are means ± standard errors of the means, with numbers of determinations in parentheses.^b On the basis of the weight of the Unisil column fraction eluted with methanol (polar lipid fraction).^c Direct chemical analysis.^d Ratio of the weights of the neutral and polar lipid fractions separated by Unisil column chromatography.^e Infection score, 5.0 ± 0.4 (4) (see references 4 and 14 for the method of determination).^f From references 14 and 29.

(10). The fraction eluted by CHCl₃ was designated the neutral lipid fraction. After elution of the neutral lipid fraction, the column was stripped with methanol (MeOH), and this fraction was designated the polar lipid fraction.

For quantitation of *P. carinii* phospholipid classes, the polar lipid fraction was subjected to analytical one-dimensional (1-D) thin-layer chromatography (TLC) on aluminum-backed Silica Gel G plates (Whatman, Kent, United Kingdom). The plates were developed in CHCl₃-acetic acid-H₂O (65:35:4, vol/vol/vol) and stained by phosphomolybdic acid (12), and phospholipid classes were quantified by plate densitometry (Shimadzu CS9000U dual-wavelength flying spot scanner; Shimadzu Scientific Instruments, Columbia, Md.). When peak areas of adjacent spots were integrated as a single component because of incomplete resolution, the individual peaks were separated by drawing a perpendicular line to the baseline of the TLC tracing. The tracing was then photocopied, the peaks were cut out and weighed, and then the percent area of each was calculated.

Polar lipid fractions were also subjected to 2-D TLC to separate and isolate individual phospholipid classes. Analytical TLC was done on 250-μm-thick Silica Gel G, and preparative TLC was done on 500-μm-thick plates (Alltech, State College, Pa.). Most separations of *P. carinii* lipids were done with CHCl₃-MeOH-28% NH₄OH (65:35:5, vol/vol/vol) in the first dimension, followed by CHCl₃-acetic acid-H₂O (65:35:4, vol/vol/vol) in the second dimension. For separation of lung control lipids, CHCl₃-MeOH-H₂O (65:25:4, vol/vol/vol) was used in the first dimension and CHCl₃-MeOH-acetic acid-H₂O (50:25:7:3, by volume) was used for development in the second dimension. Choline lipids were identified by positive staining with the Dragendorff reagent, and ninhydrin was used to identify phosphatidylethanolamine and phosphatidylserine (PS). Phosphomolybdic acid and I₂ vapor were used as general lipid stains. Lipid spots were visualized with nondestructive I₂ vapor and scraped, and the lipids were eluted with CHCl₃-MeOH (1:2, vol/vol). Total phospholipid P in the eluted samples was quantified (1).

Analyses for phosphonolipids. Inorganic P was released from total phospholipids and from phosphoryl (P-O-C) P by a differential hydrolysis protocol and was colorimetrically quantified (1, 6). The percent phosphoryl (P-C) P was calculated from the total and phosphoryl P values. The polar lipid fractions of *P. carinii*, normal lungs, and lungs of immunosuppressed uninfected rats were subjected to analysis by ³¹P nuclear magnetic resonance spectrometry (NMR) to determine the nature of P bonds in the lipid samples. The samples (10 mg of phospholipids) were dissolved in 0.5 ml of deuterated chloroform, and the analyses were done on a Bruker AMX400 400-MHz NMR spectrometer (Bruker Instruments, Billerica, Mass.) using phosphoric acid as the reference standard.

Protein analysis. Aliquots of *P. carinii* organism suspensions and lung homogenates were taken prior to the extraction of lipids. Total protein was quantified (18), and lipid values were expressed with respect to the protein contents of samples. An enzyme-linked immunosorbent assay was used to quantify SP-A with monospecific polyclonal antibodies directed against rat SP-A (14, 15).

RESULTS

Lipids and SP-A of whole rat lungs and isolated organisms. Methylprednisolone immunosuppression of rats caused an increase in the amount of lipid P per milligram of protein (*P* < 0.01). Methylprednisolone immunosuppression appeared to also increase the SP-A concentration in whole lungs (Table 1). All values obtained for normal controls (range, 0.7 to 1.3 μg of SP-A per mg of protein) were below those for the immunosuppressed uninfected lungs. The lipid contents of the lungs of

immunosuppressed rats with heavy *P. carinii* infections were not different from those of the lungs of immunosuppressed rats without infections. In contrast, the concentration of SP-A in rat lungs was doubled by the immunosuppression regimen but was increased by an order of magnitude in infected whole lungs. Compared with rat lungs, the *P. carinii* organisms contained much higher concentrations of lipids, as indicated by the lipid/protein ratio (i.e., the milligram amount of total lipid per milligram of protein in *P. carinii* organisms was higher than that in immunosuppressed uninfected lung controls [*P* < 0.015]).

Phospholipid composition. Immunosuppression not only caused an increase in the lipid content of the lung; it also caused a change in the phospholipid composition. The phosphatidylethanolamine concentration was reduced in the lungs of the corticosteroid-treated uninfected animals compared with that for normal controls (*P* < 0.01 or < 0.0025; Tables 2 and 3, respectively). The PI concentration was elevated by the immunosuppression (*P*, < 0.01 or < 0.015; Tables 2 and 3, respectively).

The values obtained for normal rat lungs by preparative TLC and chemical analysis of phospholipid P agreed well with

TABLE 2. Composition of *P. carinii carinii* and rat lung control polar lipid fractions quantified by plate densitometry of 1-D TLC separations^a

Lipid ^b	% ^c		
	<i>P. carinii</i> (6)	Lungs from:	
		Normal rats (4)	Immunosuppressed uninfected rats (4)
Origin	1.6 ± 0.4	1.4 ± 0.1	2.5 ± 0.7
PC	29.7 ± 2.1	37.3 ± 1.9	38.8 ± 2.4
PE	20.2 ± 2.3	29.7 ± 1.2	19.2 ± 2.3
PI	15.0 ± 2.3	4.9 ± 0.4	10.5 ± 1.6
SM	3.3 ± 0.6	4.1 ± 0.2	9.0 ± 1.6
PS	12.8 ± 1.9	4.6 ± 0.5	2.9 ± 1.6
LPC	2.3 ± 0.4	0.8 ± 0.2	0.9 ± 0.4
PG	10.0 ± 1.7	12.7 ± 0.6	9.9 ± 3.3
CL	2.8 ± 0.6	2.6 ± 0.5	2.7 ± 0.5
Other	2.0 ± 0.3	1.6 ± 0.4	3.8 ± 0.8

^a Phosphomolybdic acid general lipid stain.^b PE, phosphatidylethanolamine; LPC, lysophosphatidylcholine; PG, phosphatidylglycerol; CL, cardiolipin.^c The data are means ± standard errors of the means. The numbers of determinations are indicated in parentheses.

TABLE 3. Phospholipid composition of purified preparations of *P. carinii carinii* mixed life cycle stages and lung controls determined by chemical analysis of lipids isolated by 2-D TLC^a

Lipid ^b	% of total phospholipid phosphorus				Lungs from immunosuppressed uninfected rats	% Phosphonolipids in <i>P. carinii</i> ^f
	<i>P. carinii</i> ^c	Normal rat lungs				
		This study	Reference 2 ^d	Surfactant ^e		
PC	39.3 ± 2.4 (10)	46.6 ± 1.3 (4)	44.9	78.8	49.9 ± 2.6 (4)	1.2 ± 1.8 (11)
PE	14.5 ± 0.8 (10)	20.4 ± 0.8 (4)	22.2	4.3	12.3 ± 1.6 (4)	3.2 ± 2.3 (6)
PI	11.3 ± 0.8 (8)	2.3 ± 0.4 (4)	3.9	3.3	4.0 ± 0.4 (4)	2.8 ± 0.7 (5)
SM	10.0 ± 1.9 (10)	11.7 ± 0.6 (4)	10.0	1.5	8.9 ± 0.9 (4)	0.2 ± 1.9 (3)
PS	9.5 ± 1.6 (8)	6.8 ± 0.4 (4)	9.0	1.5	8.3 ± 0.5 (4)	2.2 ± 0.7 (6)
LPC	6.3 ± 1.3 (10)	2.4 ± 0.5 (4)	1.3	1.5	4.9 ± 0.4 (4)	2.6 ± 3.8 (5)
PG	5.6 ± 0.9 (10)	4.4 ± 0.5 (4)	2.2	9.2	6.6 ± 0.5 (4)	2.8 ± 0.7 (5)
CL	3.9 ± 0.7 (10)	5.4 ± 1.1 (4)	1.1	ND ^g	5.2 ± 0.4 (4)	−1.2 ± 4.7 (6)
Total phospholipid						1.0 ± 1.6 (8)

^a The data from this study are means ± standard errors of the means, with numbers of determinations in parentheses.

^b PE, phosphatidylethanolamine; LPC, lysophosphatidylcholine; PG, phosphatidylglycerol; CL, cardiolipin.

^c When PI and PS were not sufficiently resolved by 2-D TLC, the combined spot was analyzed to calculate the percent phospholipid P of the well-resolved lipids, but the values for PI and PS were excluded in calculating their percentages of total phospholipid P.

^d Wistar strain rats.

^e From reference 23; male Sprague-Dawley rats were used.

^f In some analyses, samples of isolated minor lipids were pooled.

^g ND, not detected.

the data obtained by other investigators using similar methods (Table 3). The values obtained for phospholipid composition of lung controls and for *P. carinii* by the two methods in this study were in general agreement. However, the absolute values obtained by the two analytical techniques were different. The TLC densitometry data (Table 2) represent weight percent compositions, whereas the 2-D TLC and phospholipid P analyses reflect molar concentrations (Table 3), although the value for cardiolipin was not adjusted to account for the presence of two P atoms in this lipid. Discrepancies in the results obtained by the two standardly accepted lipid analytical procedures may be due to incomplete recovery of lipids eluted from TLC plates prior to P analysis, errors in background subtraction during TLC plate densitometry integration, or differences in the lipids in the biological samples used for the different sets of analyses. However, the most likely basis for the differences is that differential staining of sphingolipids and other lipids by phosphomolybdate occurred (12). No other general lipid stains were used in the present study to determine if other stains gave results more consistent with the phospholipid P values. The data obtained by phospholipid P analysis are considered more useful for comparison with values currently available in the literature (Table 3).

The composition of *P. carinii* phospholipids grossly reflected those of lungs from normal and immunosuppressed uninfected rats, but the amount of PC was lower ($P < 0.02$ or < 0.015 ; Table 2 and 3, respectively) and the amount of PI was higher ($P < 0.001$; Table 3) in the organism. Polyphosphoinositides were not measured in this study. The lipids in the lung surfactant contain about 80% PC (Table 3), a much higher proportion than that in the organism; thus, *P. carinii* has a phospholipid composition distinct from that of its immediate environment.

Differential hydrolysis of total lipids as well as individual phospholipids isolated by 2-D TLC indicated that phosphonolipids were either present only in very low concentrations or absent. The absence of phosphonolipids was shown by ³¹P NMR analyses of the total polar lipids (Fig. 1). A peak at chemical shift for P-C, about 20 ppm downfield from P-O-C, was not detected in 512 scans of the polar lipid fractions from *P. carinii* organisms or immunosuppressed uninfected lung controls or in 100 scans of normal lung controls.

DISCUSSION

Changes in lung lipids of immunosuppressed uninfected rats were previously noted by Sheehan et al. (23). However, the values obtained in that study were different from those obtained in the present study. For example, the previous report indicated that untreated (not immunosuppressed) control postlavage lung tissue contained only 18 nmol of total phospholipids per mg of protein. The phospholipid contents of postlavage immunosuppressed uninfected controls and *P. carinii*-infected lungs were reported to be 200 and 83 nmol/mg of protein, respectively (23). As a consequence of the low levels of phospholipids in postlavage normal lung tissue, these workers concluded that immunosuppression caused an 11-fold increase in the phospholipid content of the lung and that the phospho-

FIG. 1. NMR spectrum of the *P. carinii carinii* total phospholipid fraction demonstrating the phosphoryl (P-O-C) bonds present in the phospholipids. Phosphonyl (P-C) bonds, at about 20 ppm, were not detected in 512 accumulated scans.

lipid content of *P. carinii*-infected lungs was less than half of that in immunosuppressed controls. The phospholipid contents of normal and immunosuppressed uninfected whole-lung tissues analyzed in the present study were 159 and 258 nmol/mg of protein, respectively. Thus, the effect of immunosuppression on whole-lung phospholipid content was not as great (an increase of ca. 62%) as that in postlavage lungs (23). In the present study, the phospholipid content of *P. carinii*-infected whole lungs (infection scores $> +5$) was not different from that in whole lungs of immunosuppressed uninfected rats. Although we used rats of a different strain and sex, used methylprednisolone instead of dexamethasone immunosuppression, did not treat the rats with antibacterial or antifungal drugs as Sheehan et al. did, and did not lavage (but perfused) the lungs before extracting for lipids, such a large difference in values for phospholipid content (phospholipid/protein ratio) between the two studies was unexpected.

The concentrations of both total lipids and total phospholipids in *P. carinii* organisms were at least twice those in the lung tissues of normal rats with respect to protein content. These data are consistent with the observation that *P. carinii* can readily take up fluorescent analogs of complex phospholipids in vitro (24), suggesting the ease with which it could scavenge host pulmonary surfactant lipid. Evidence for de novo lipid synthesis of several lipid components by the organisms has recently been demonstrated (7, 10, 11). Endogenous lipid synthesis in the organism would also contribute to its high lipid content. The organism is capable of storing large amounts of lipids. The presence of cytoplasmic osmiophilic lipid droplets, the sites of lipid storage, have been shown by electron microscopy (28), which supports the conclusion that the organism is enriched in lipids. Because the organisms are enriched in lipids, we further suggest that the lowered phospholipid content determined for postlavage *P. carinii*-infected lungs by Sheehan et al. (23) could have resulted from the removal of much of the lipid-laden organisms from the lungs by bronchoalveolar lavaging of the rats prior to analysis of the lungs.

Electron micrographs of heavily infected lungs demonstrate that *P. carinii*, tightly adhered to one another and stacked in layers of three or four organisms, form bridges or caps over epithelial type II cells. Under these caps, material with structures diagnostic of the lung surfactant appears to be trapped in pockets under the organism layer (16a). This suggests that surfactant proteins secreted by type II epithelial cells may accumulate there. Although SP-A and SP-D tightly bind to *P. carinii* surfaces (17, 29), the organism probably does not internalize and metabolize substantial quantities of it. However, the high lipid concentrations in *P. carinii* and the rapid uptake of intact fluorescent phospholipids by the organism (24) support the notion that a large proportion of surfactant lipids become part of the organisms.

The observation that lung surfactant phospholipids decrease but SP-A increases in BALF of infected lungs (27) could be explained by combinations of the following: (i) dislodging of SP-A trapped under layers of organisms that is washed into BALF during lavaging, (ii) removal of the lipid-laden organisms prior to the analysis of the lavage fluid, (iii) catabolism of fatty acids to CO₂ by *P. carinii* as a major energy source (18a), and (iv) phospholipase activity associated with the pathogen (16, 24). Phospholipase A activity has been detected in *P. carinii* cell suspensions during short-term incubations with fluorescent phospholipid analogs (23a).

These studies, together with other observations discussed above, suggest that long-term surfactant replacement therapy for *P. carinii* pneumonia may serve to provide more lipids for *P. carinii*, thereby supporting its proliferation. Furthermore,

the earlier studies on cortisone- and dexamethasone-treated animals (16, 23), together with the present report on the methylprednisolone-immunosuppressed *P. carinii* rat model, have important implications for the use of steroids for *P. carinii* pneumonia therapy. Following the administration of drugs to eliminate *P. carinii*, there is an inflammatory response in the lung characterized by decreased blood oxygenation, which is alleviated by steroid therapy (5). It is possible that the lysis of the parasites in response to the drugs releases lipids that contribute to the inflammatory response since several lipids (e.g., sphingolipids, ether lipids, and eicosanoids) are known to be cytotoxic or have potent bioactivities. However, if steroids themselves increase lung surfactant lipid production, it is possible that the proliferation of the pathogen would be enhanced by steroids; thus, extended administration of steroids could have adverse consequences.

The relatively high level of PC in *P. carinii* is not surprising, since parasites in general scavenge host lipids and the lung surfactant is characterized by high dipalmitoyl PC concentrations. However, our results demonstrate that the organism has relatively less PC, and more PI, than is present in the lung surfactant, the environment in which the parasite resides (Table 3). This study presents quantitative data demonstrating that the phospholipid composition of *P. carinii* differs from those of the lung controls (normal and immunosuppressed) and surfactant phospholipids, differences which, we believe, were detectable because of the purity of the organism preparation.

It has been reported that sphingomyelin (SM) is elevated in the BALF obtained from *P. carinii*-infected corticosteroid-immunosuppressed rats (25). Consistent with that study is the demonstration that SM synthesis (analyzed by [³H]choline incorporation into the phospholipid) in type II pneumocytes isolated from corticosteroid-immunosuppressed *P. carinii*-infected rats is markedly elevated (22). In the present study, we did not observe an increase in SM concentration in whole-lung lipids in response to steroid treatment and did not analyze the phospholipid composition of *P. carinii*-infected whole lungs. If SM concentrations in infected whole lungs increased as a specific response to *P. carinii* infection, it would be expected that the SM concentration of whole-lung lipids might vary according to the level of infection.

Cardiolipin (diphosphatidylglycerol) is normally found in eukaryotic cells restricted to the mitochondria; thus, tissues with numerous mitochondria have elevated concentrations of cardiolipin. Another mitochondrial lipid is ubiquinone (coenzyme Q), which functions in the respiratory chain, although it also occurs in other cellular membranes (26). It is interesting that the cellular concentration of ubiquinone in *P. carinii* was found at the lower end of the range of values typically found for aerobic organisms, suggesting that the organism may not undergo strictly aerobic metabolism (11). The present study indicates that unlike ubiquinone, the cardiolipin level in *P. carinii* is comparable to that generally found in other cells and in aerobic protozoa such as *Paramecium tetraurelia* (21). Electron microscopy demonstrates that mitochondria in *P. carinii* are not unusual and that they have extensive inner-membrane cristae (14, 28).

If phosphonolipids were present in *P. carinii*, they would be expected to occur among the ethanolamine phospholipids, where they usually occur in organisms that have these lipids (21). The values we obtained for phosphonolipids detectable in the total lipids, and in the individual phospholipid classes, suggested that they were not present. Analyses by NMR also indicated that they were absent; hence, we conclude that the lipids of *P. carinii* are devoid of phosphonolipids.

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