Head group precursors modify phospholipid synthesis in *Schistosoma mansoni*

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Abstract The two predominant phospholipids in schistosomula of *Schistosoma mansoni* are phosphatidylcholine (PC) and phosphatidylethanolamine (PE) which are found in a molar ratio of 0.52 (PE/PC). The incorporation of four fatty acids (arachidonic, myristic, oleic, and palmitic) and glycerol into phospholipids of schistosomula was measured. In two different media (one containing ethanolamine, the other without), all four fatty acids were predominantly incorporated into PC with a PE/PC ratio of -0.1 in a 90-min label. After a 24-h chase, PC remained the predominant labeled phospholipid but the fatty acid-labeled PE/PC ratio increased slightly, the specific activity of labeled neutral lipids decreased, and the specific activity of labeled PE increased. Glycerol was incorporated with a ratio of 0.55 in the presence of ethanolamine but only 0.19 in its absence. Schistosomula also incorporate fatty acids into phosphatidylmonomethylethanolamine (PMME) and phosphatidyldimethylethanolamine (PDME) at rates intermediate to that into PE and PC in the presence of the respective head group precursor; this incorporation was inhibited by choline. Relative to PC, oleic acid is incorporated into PE, PMME, and PDME at rates higher than for palmitic acid. These results suggest that schistosomula possess acyltransferase(s) with head group specificity and that acyl chains are transferred from neutral lipids to phospholipids over time. Furlong, S. T., and J. P. Caulfield. Head group precursors modify phospholipid synthesis in *Schistosoma mansoni*. *J. Lipid Res.* 1991. 32: 703-712.

Supplementary key words schistosomula • fatty acids • choline analogs • ethanolamine

Schistosomiasis is a human disease caused by the trematode *Schistosoma mansoni* which affects greater than 200 million people worldwide (1). Recent studies have suggested that lipids may defend the parasite from host immune response (2–5). However, lipids and lipid precursors must be acquired from the host because the parasite does not make fatty acids or sterols de novo (6). Despite the importance of lipids in the parasite's life cycle, little information is available about the parasite's mechanisms for regulating lipid synthesis or uptake of lipid precursors.

The two major classes of phospholipids found in schistosomula of *Schistosoma mansoni* are phosphatidylcholine (PC) and phosphatidylethanolamine (PE) (7, 8). The parasites incorporate radiolabeled precursors into these phospholipids, i.e., choline into PC, ethanolamine into PE, and fatty acids into both. Our previous studies have shown that palmitic and oleic acids are incorporated primarily into PC with much less labeling of PE, PS, and PI (2, 9). Although these results are similar to results in mammalian cells (10–15), all previous studies of schistosome lipid metabolism have been carried out in nondefined media or defined media that differed significantly from physiological concentrations of lipid precursors (2, 6, 16–18). Therefore, exogenous phospholipid head group precursor concentrations may have influenced the results of these studies. In other organisms, such as yeast (19, 20), and in mammalian cells (21–24), lipid synthesis is clearly affected by head group concentration. Furthermore, fatty acid incorporation into PE by schistosomula in the presence of ethanolamine (17) was reported to be higher relative to PC than in the absence of ethanolamine (2, 9).

In the present study, we have measured the effect of various head group precursors, particularly ethanolamine, on the incorporation of fatty acids and glycerol label into the major lipid classes. Studies were performed in a standard defined culture medium, a modified medium containing physiological concentrations of phospholipid head group precursors, or standard media containing up to 10x physiological concentrations of ethanolamine.

Four labeled fatty acids ([9,10-3H]palmitic acid, [9,10-3H]oleic acid, [9,10-3H]myristic acid or [5,6,8,9,11,12,14,15-3H]arachidonic acid) and [2-3H]glycerol were chosen as precursors. Palmitic acid and oleic acid are the most...
prevailing fatty acids in the organism (25, 26). Myristic acid and palmitic acid are important for other synthetic pathways such as protein acylation (27, 28). Arachidonic acid is a precursor for biologically active metabolites produced by the parasite (29). These four fatty acids constitute approximately 50% of the total found in the organism (25, 26). For comparison to fatty acid labeling, worms were also labeled with [3H]glycerol.

The influence of choline analogs on fatty acid incorporation was also studied. Ethanolamine and choline differ only in the number of methyl substitutions. Head groups with an intermediate number of methyl groups such as monomethylethanolamine (MME) and dimethylethanolamine (DME) may be incorporated into the respective analog containing phospholipids, thereby modifying the parasite's lipid content. Furthermore, the relative rate at which fatty acids are incorporated into these analog-containing phospholipids may be between that of PE and PC if the number of methyl substitutions on the head group influences rate of acylation.

The major questions asked by this study are: first, to what extent are fatty acid and glycerol incorporation into worm phospholipids influenced by head group precursor concentration? Second, can supraphysiological concentrations of ethanolamine increase PE synthesis beyond that measured in the presence of physiological concentrations of ethanolamine? Third, will MME and DME be incorporated into phospholipids and will these analogs inhibit PE or PC synthesis or themselves be inhibited by high concentrations of choline? These studies have focused on PE, PC, and choline analogs due to their similarities in head group structure, because PC and PE are the most abundant phospholipids in the organism, and because the de novo synthetic pathways for these phospholipids are similar (22).

**Materials and Methods**

**Culture conditions**

Cercariae (Puerto Rican strain) obtained from infected snails were transformed mechanically by vortexing. The resulting bodies and tails were separated on a Percoll density gradient. The bodies were washed three times and resuspended in RPMI 1640 at 37°C for 3 h to allow complete transformation (30). Schistosomula were cultured overnight at a concentration of 1 × 10⁶ organisms/ml in either standard or modified (see below) medium RPMI 1640 containing 0.1% BSA, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C under an atmosphere of 5% CO₂ and 95% O₂. Viability, as assessed by examining the cultured organisms for head and torso movement and flame cell activity, was identical in either culture medium after 24 h and was greater than 95% for all experiments.

To compare the effect of culture medium polar head group precursors on labeled fatty acid and glycerol incorporation, two types of RPMI were used: 1) Standard RPMI-Powdered RPMI from GIBCO (Grand Island, NY) reconstituted with deionized water. Standard RPMI does not contain ethanolamine, but does contain choline, serine, and inositol as shown in Table 1. 2) Modified RPMI-A custom formulated RPMI was purchased from Sigma Chemical Company (St. Louis, MO) (Product #R6882 lot #128F4625-1) that contained no choline, serine, inositol, glutamine, or sodium bicarbonate. Lipid precursors were then added to approximate concentrations found in human serum (Table 1). Both media contained 2 mM glutamine, 24 mM sodium bicarbonate, and 10 mM HEPES at pH 7.4.

**Metabolic labeling of schistosomula**

All metabolic labeling experiments were conducted on schistosomula that had been cultured overnight. To compare the incorporation of labeled precursors in modified medium and standard RPMI and to determine the turnover of fatty acid precursors among lipid classes, schistosomula (5 × 10⁴) were incubated for 90 min with tritium-labeled precursor in 1 ml of medium at 37°C under 5% CO₂ with one of the following: 200 μCi [9,10-³H]myristic acid (sp act 47.5 Ci/mmol, Amersham, Arlington Heights, IL); 100 μCi [9,10-³H]palmitic acid (sp act 30 Ci/mmol, New England Nuclear, Boston, MA); 100 μCi [9,10-³H]oleic acid (sp act 10 Ci/mmol, New England Nuclear); 10 μCi[5,6,8,9,11,12,14,15³H]arachidonic acid (sp act 100 or 240 Ci/mmol, New England Nuclear); and 100 μCi [2-³H]glycerol (sp act 40 Ci/mmol, New England

**Table 1. Concentration of phospholipid head group precursors in human serum and culture media**

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Normal Human Serum</th>
<th>Standard RPMI*</th>
<th>Modified Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choline</td>
<td>14-110 (38-41)</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>Ethanolamine</td>
<td>11.7-11.8 (42, 43)</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Serine</td>
<td>101-144 (43-46)</td>
<td>285</td>
<td>100</td>
</tr>
<tr>
<td>Inositol</td>
<td>30 (47, 48)</td>
<td>194</td>
<td>20</td>
</tr>
</tbody>
</table>

All values are expressed as micronolar concentrations. Numbers in parentheses refer to reference from which values were taken.

*Values shown in Standard RPMI are from the GIBCO catalog.
Precursors were dried from organic solvent into the bottom of a 15-ml glass centrifuge tube, resuspended in an aliquot of culture medium containing 0.1% BSA, vortexed vigorously for 1 min, and then sonicated in a bath sonicator for 10 min. At the end of the incubation with precursor, organisms were washed three times with fresh medium containing 0.1% BSA. For organisms labeled with arachidonic acid, oleic acid, or palmitic acid, one half of the labeled schistosomula were cultured for an additional 24 h in fresh medium while the remainder were immediately frozen for later analysis (described below). For organisms labeled with glycerol or myristic acid the entire sample was frozen at the end of the 90-min incubation and turnover was not examined.

For experiments to test the influence of different concentrations of ethanolamine, MME, or DME on fatty acid incorporation into phospholipids, 5000 schistosomula that had been cultured overnight in standard RPMI were placed in each well of a 24-well flat-bottom culture plate containing from 0-200 μM ethanolamine, MME, or DME, or 200 μM of analog plus 200 μM choline. After a 3-h preincubation, 25 μCi of [3H]palmitic acid (sp act 30 Ci/mmol, New England Nuclear) or 25 μCi [3H]oleic acid (10 Ci/mmol, New England Nuclear) was added to each well and the incubation was carried out for an additional 7 h. At the end of the incubation, labeled parasites were removed from each well to 15-ml centrifuge tubes containing fresh medium and washed 2 x in RPMI containing 0.1% BSA. Labeled lipids were extracted and quantitated as below.

**Extraction, separation, and quantitation of labeled lipids**

Procedures for extracting, separating, and quantitating lipids from schistosomula have been described previously (2, 8, 9). In brief, lipids were extracted from parasites and culture medium with chloroform-methanol 2:1 (31). Phospholipids were separated by two methods on silica columns with a Waters automated gradient HPLC system: Method 1) a 25 cm by 4.0 mm steel column packed with 5 μm Econosphere silica (Alltech Applied Sciences, State College, PA) with a mobile phase of acetonitrile-methanol-85% phosphoric acid 130:6:1.5 flowing at either 1 or 1.25 ml/min (9, 32); Method 2) a 25 cm by 4.6 mm steel column packed with 5 μm Lichrosphere silica (Supelco, Bellefonte, PA) was eluted with a linear gradient of hexane-isopropanol-water 44:50:6 to 54:40:6 at 1.5 ml/min for 20 min and held at the final proportions for an additional 5 min (33). The linear gradient was then reversed in 5 min to the original conditions. Method 1 was used for most studies and separated all the major phospholipid classes but did not resolve labeled PE from PMME. PE and PMME were separated using method 2, however, and this method was used for most of the experiments with choline analogs. With both methods radioactivity was quantitated by an on-line Radiomatic model BD radioactivity detector with Flo-Scint 1 scintillation cocktail (Radiomatic Instruments and Chemical Co., Tampa, FL) at a split ratio of 1:3 (eluents:cocktail) and calibrated with [3H]dipalmitoylphosphatidylcholine (sp act 58 Ci/mmol, New England Nuclear). Lipids were identified by comparison to the retention times of unlabeled standards detected by a Waters model 441 UV detector at 214 nm. Standards were purchased from Avanti Polar Lipids (Birmingham, AL). Data were collected with either a Nelson Model 760 or 960 interface and integrated with Nelson Analytical Software on a Hewlett-Packard Vectra computer.

Results for biosynthetic labeling experiments were expressed both as a specific activity (moles of precursor incorporated/mole of lipid phosphorus) and as a percentage of label in each lipid class. Moles of precursor incorporated were determined by converting the radioactivity measurements to moles based on the specific activity of the precursor added to the medium. The moles of total lipid phosphorus were calculated from the HPLC UV detector response (9). Specific activity was the ratio of these two values. Percentage of each lipid class was calculated from the total radioactivity eluted from the HPLC column.

A paired Wilcoxon’s test was used for statistical analyses.

**RESULTS**

**Distribution of turnover of fatty acids among phospholipid classes**

Standard RPMI 1640 contains concentrations of phospholipid head group precursors that differ markedly from those present in human serum (Table 1). To test the possibility that head group precursor concentrations influence the relative rate of fatty acid incorporation or turnover among phospholipid classes, schistosomula were cultured in either standard RPMI 1640 or a medium modified to contain physiological concentrations of these precursors (Table 2). In both standard and modified media >90% of all four labeled fatty acids were incorporated into neutral lipids (eluting in the solvent front) and PC after 90 min of continuous labeling (Table 2). For phospholipids, in either culture medium, with any of the four fatty acid precursors, only PC and PE were labeled appreciably. Further, the specific activity of PC was approximately an order of magnitude higher than PE. LPC labeling with [3H]arachidonic acid and [3H]oleic acids and phosphatidylserine (PS) labeling with [3H]arachidonic acid and [3H]myristic acid were detected variably. [3H]Palmitic acid and [3H]myristic acid labeling of LPC, PS labeling with [3H]palmitic acid and [3H]oleic acid and phosphatidylinositol (PI) with all precursors was 1% or less of the total radioactivity. Although the labeling...
Dominant labeled phospholipids were PC and PE and the proportion of radioactivity in the phospholipids was primarily dependent on the fatty acid precursor but could be caused by release of neutral lipid into the culture medium as previously described (9) or transfer of acyl chains to proteins and phospholipids. The specific activity of PE increased in all labels and of the oleic acid-labeled PC demonstrates that acyl chains are transferred into the phospholipids from the neutral lipids.

In addition to continuous labeling, acyl chain turnover was also quantitated for schistosomula labeled with arachidonic acid, palmitic acid, and oleic acid (Table 3). The total radioactivity fell for all labels in either medium, by approximately 40% for arachidonic and oleic acids and approximately 70% for palmitic acid. This loss was mainly due to a decrease in the specific activity of the neutral lipids of approximately 50% for arachidonic acid, 40% for oleic acid, and 85% for palmitic acid.

### TABLE 2. Incorporation of labeled fatty acids and glycerol into neutral lipids and phospholipids in modified or standard medium

<table>
<thead>
<tr>
<th>Label</th>
<th>Medium</th>
<th>NL(%)</th>
<th>PE(%)</th>
<th>PC(%)</th>
<th>PE/PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>MOD</td>
<td>55.7 ± 5.4</td>
<td>5.0 ± 1.1</td>
<td>37.5 ± 4.9</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(7.5 × 10⁴)</td>
<td>(6.7 × 10⁴)</td>
<td>(5.1 × 10⁴)</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>STD</td>
<td>43.9 ± 10.6</td>
<td>5.0 ± 0.7</td>
<td>47.6 ± 10.1</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4.5 × 10⁴)</td>
<td>(3.1 × 10⁴)</td>
<td>(4.5 × 10⁴)</td>
<td></td>
</tr>
<tr>
<td>OA</td>
<td>MOD</td>
<td>70.6 ± 9.9</td>
<td>3.7 ± 1.4</td>
<td>23.2 ± 8.7</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.9 × 10⁴)</td>
<td>(6.3 × 10⁴)</td>
<td>(4.2 × 10⁴)</td>
<td></td>
</tr>
<tr>
<td>OA</td>
<td>STD</td>
<td>74.7 ± 6.2</td>
<td>2.6 ± 1.0</td>
<td>20.3 ± 5.6</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2.7 × 10⁴)</td>
<td>(1.1 × 10⁴)</td>
<td>(7.7 × 10⁴)</td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>MOD</td>
<td>77.1 ± 1.6</td>
<td>0.7 ± 0.1</td>
<td>19.9 ± 1.3</td>
<td>0.03</td>
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<tr>
<td></td>
<td></td>
<td>(6.2 × 10⁴)</td>
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<td>(1.6 × 10⁴)</td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>STD</td>
<td>80.1 ± 9.9</td>
<td>0.4 ± 0.1</td>
<td>17.0 ± 10.6</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(7.4 × 10⁴)</td>
<td>(4.5 × 10⁴)</td>
<td>(1.9 × 10⁴)</td>
<td></td>
</tr>
<tr>
<td>MA</td>
<td>MOD</td>
<td>68.8 ± 5.7</td>
<td>3.8 ± 1.0</td>
<td>27.3 ± 5.0</td>
<td>0.14</td>
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<tr>
<td></td>
<td></td>
<td>(9.0 × 10⁴)</td>
<td>(1.2 × 10⁴)</td>
<td>(3.1 × 10⁴)</td>
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<tr>
<td>MA</td>
<td>STD</td>
<td>70.5 ± 10.9</td>
<td>1.1 ± 0.6</td>
<td>28.1 ± 10.5</td>
<td>0.04</td>
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<td></td>
<td></td>
<td>(2.4 × 10⁴)</td>
<td>(2.1 × 10⁴)</td>
<td>(6.3 × 10⁴)</td>
<td></td>
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<tr>
<td>GLY</td>
<td>MOD</td>
<td>50.3 ± 16.5</td>
<td>17.7 ± 9.4</td>
<td>32.0 ± 14.9</td>
<td>0.55</td>
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<tr>
<td></td>
<td></td>
<td>(2.8 × 10⁴)</td>
<td>(1.2 × 10⁴)</td>
<td>(2.8 × 10⁴)</td>
<td></td>
</tr>
<tr>
<td>GLY</td>
<td>STD</td>
<td>60.1 ± 16.7</td>
<td>6.4 ± 0.8</td>
<td>33.6 ± 8.7</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.3 × 10⁴)</td>
<td>(1.9 × 10⁴)</td>
<td>(1.1 × 10⁴)</td>
<td></td>
</tr>
</tbody>
</table>

- **AA**: Arachidonic acid
- **OA**: Oleic acid
- **PA**: Palmitic acid
- **MA**: Myristic acid
- **GLY**: Glycerol
- **NL**: Neutral lipids
- **PE**: Phosphatidyl ethanolamine
- **PC**: Phosphatidyl choline

Distribution of radioactivity among the major lipid classes after labeling in either standard RPMI 1640 (STD) or modified medium (MOD). Schistosomula were incubated with indicated precursors for 90 min, extracted with chloroform-methanol, and labeled lipids were separated by HPLC and quantitated as described in Methods. Each value is the mean and standard deviation of three separate experiments that measured the radioactivity eluted from the HPLC column measured in three separate experiments. Numbers in parentheses are the mean of the specific activity from the same three experiments and represent mole of label incorporated into each lipid class per mole of total lipid phosphorus. GLY, glycerol; NL, neutral lipids; AA, arachidonic acid; OA, oleic acid; PA, palmitic acid; MA, myristic acid. The PE/PC molar ratio of unlabeled phospholipid in schistosomula is 0.52 (Ref. 8).
measured from the total phospholipids of unlabeled worms (8).

[\textsuperscript{3}H]Glycerol is incorporated into PE at a higher rate in modified than standard medium

For comparison to the fatty acid labels, schistosomula were also labeled with [\textsuperscript{3}H]glycerol for 90 min in standard and modified medium. Glycerol, like the fatty acid precursors, is incorporated predominantly into neutral lipids and PC. However, the percentage of total label incorporated into PE was higher in modified than standard medium (17% vs. 6%). The PE/PC ratio determined from glycerol incorporation was 0.19 in standard medium, and 0.55 in modified medium. The latter value was close to the PE/PC ratio of 0.52 of total lipids (Table 2). Thus incorporation of [\textsuperscript{3}H]glycerol into PE was increased by exogenous ethanolamine more than that of the fatty acid precursors tested. In either medium, incorporation of [\textsuperscript{3}H]glycerol into PI, PS, and LPC was below detection limits.

Exogenous ethanolamine has little effect on the PE/PC ratio of fatty acid-labeled organisms

Labeling of schistosomula with fatty acids in ethanolamine-modified medium only slightly increased the PE/PC ratio (Table 2). We also measured whether supraphysiological concentrations of ethanolamine could increase the PE/PC ratio further in oleic acid- and palmitic acid-labeled organisms. Palmitic acid and oleic acid were chosen because they are the most abundant fatty acids in the organism. As shown in Fig. 1A, [\textsuperscript{3}H]palmitic acid incorporation into PE doubled between 0 and 100 \textmu M ethanolamine and showed no further increase at 200 \textmu M. Even at the highest concentration of ethanolamine tested, however, PC was the predominant labeled phospholipid. Fig. 1B shows that the proportion of palmitic acid-labeled PE relative to palmitic acid-labeled PC increased by a factor of two but reached a maximum at 20 \textmu M ethanolamine where incorporation of palmitic acid into PE was less than 3% that of PC. It is noteworthy that saturation occurred at close to physiological concentrations of ethanolamine. Similar results were observed in organisms labeled with [\textsuperscript{3}H]oleic acid. As shown in Table 2, the PE/PC ratio is higher in [\textsuperscript{3}H]oleic acid-labeled organisms than for the corresponding [\textsuperscript{3}H]palmitic acid-labeled organisms in medium containing 12 \textmu M ethanolamine. In 200 \textmu M ethanolamine, oleic acid incorporation into PE was also slightly greater than found in physiological concentrations of ethanolamine (6.4% vs. 3.7%). For either [\textsuperscript{3}H]palmitic acid- or [\textsuperscript{3}H]oleic acid-labeled organisms, the increased PE labeling due to supraphysiological concentrations of ethanolamine was abolished by the presence of 200 \textmu M choline. These results showed that the low ratio of fatty acid incorporation into PE compared to PC was not due to the concentration of ethanolamine in the culture medium.

Choline analogs alter fatty acid incorporation into phospholipids

MME and DME contain one and two methyl groups, respectively, compared with three methyl groups for choline. To determine whether schistosomula could incorporate choline analogs into phospholipids, the organisms

<table>
<thead>
<tr>
<th>Label</th>
<th>Medium</th>
<th>NL(%)</th>
<th>PE(%)</th>
<th>PG(%)</th>
<th>PE/PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>MOD</td>
<td>34.6 ± 12.6 (3.1 × 10\textsuperscript{-6})</td>
<td>11.3 ± 1.4 (9.4 × 10\textsuperscript{-6})</td>
<td>51.0 ± 10.6 (4.2 × 10\textsuperscript{-6})</td>
<td>0.22</td>
</tr>
<tr>
<td>AA</td>
<td>STD</td>
<td>35.7 ± 9.4 (2.7 × 10\textsuperscript{-6})</td>
<td>11.7 ± 4.7 (7.9 × 10\textsuperscript{-6})</td>
<td>47.3 ± 4.9 (3.4 × 10\textsuperscript{-6})</td>
<td>0.25</td>
</tr>
<tr>
<td>OA</td>
<td>MOD</td>
<td>39.6 ± 14.3 (6.1 × 10\textsuperscript{-6})</td>
<td>11.8 ± 3.5 (1.4 × 10\textsuperscript{-6})</td>
<td>45.8 ± 10.7 (5.1 × 10\textsuperscript{-6})</td>
<td>0.26</td>
</tr>
<tr>
<td>OA</td>
<td>STD</td>
<td>51.0 ± 3.5 (1.3 × 10\textsuperscript{-6})</td>
<td>5.2 ± 1.2 (1.5 × 10\textsuperscript{-6})</td>
<td>40.8 ± 2.3 (1.1 × 10\textsuperscript{-6})</td>
<td>0.13</td>
</tr>
<tr>
<td>PA</td>
<td>MOD</td>
<td>35.6 ± 7.8 (8.5 × 10\textsuperscript{-6})</td>
<td>2.1 ± 1.5 (5.2 × 10\textsuperscript{-6})</td>
<td>60.5 ± 7.2 (1.5 × 10\textsuperscript{-6})</td>
<td>0.03</td>
</tr>
<tr>
<td>PA</td>
<td>STD</td>
<td>34.3 ± 2.8 (1.0 × 10\textsuperscript{-6})</td>
<td>4.0 ± 2.3 (8.2 × 10\textsuperscript{-6})</td>
<td>59.2 ± 5.5 (1.9 × 10\textsuperscript{-6})</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Distribution of radioactivity among the major lipid classes 24 h after labeling in either standard RPMI 1640 or modified medium. Schistosomula were labeled in indicated precursors for 90 min, washed, resuspended in fresh medium, and cultured for an additional 24 h. Percentages shown represent mean percent and standard deviation of total radioactivity eluted from the HPLC column measured in three separate experiments. Numbers in parentheses are moles incorporated into each lipid class per mole of lipid phosphorus. Abbreviations as in Table 2.
were labeled with palmitic acid or oleic acid in media containing 0–200 μM MME or DME. In the presence of the choline analogs, both fatty acid labels were incorporated mainly into neutral lipids and PC with much less into PE (Figs. 2A–C). However, the fatty acids were also incorporated into novel lipids that were not present in worms cultured in the absence of the analogs. These new lipids had retention times that corresponded to phosphatidylmonomethylethanolamine (PMME) for worms cultured in MME containing media (Fig. 2A and B) and phosphatidyldimethylethanolamine (PDME) in DME containing media (Fig. 2C).

![Graph A](image1)

**Fig. 1.** Effect of ethanolamine and DME concentration on incorporation of [3H]palmitic acid into phospholipids. Schistosomula were incubated overnight in standard RPMI containing 0–200 μM ethanolamine in wells of a 24-well culture dish. Organisms were labeled the following day with [3H]palmitic acid as described in the text. Lipids were extracted, separated by HPLC method I and quantitated. As shown in A, incorporation into PE increased twofold at the highest concentration of ethanolamine. Fig. 1B shows that the increase in incorporation into PE relative to palmitic acid-labeled PC reached a maximum at approximately 20 μM and that at the highest concentration of ethanolamine tested the proportion of labeled PE remained small relative to PC. As shown in C, incorporation of [3H]palmitic acid into PDME reached a maximum at the same concentration as that for [3H]palmitic acid into PE. Values are mean ± standard deviation for three separate experiments.

![Graph B](image2)

![Graph C](image3)

**Fig. 2.** Separation of [3H]palmitic acid- or [3H]oleic acid-labeled phospholipids from schistosomula cultured with choline analogs. Schistosomula were labeled with either [3H]palmitic acid (A) or [3H]oleic acid (B, C) in the presence of either 200 μM MME (A, B) or 200 μM DME (C). Labeled PMME was present in organisms cultured in MME and labeled PDME in organisms cultured in DME. Labeled PMME and PDME were not observed in the absence of analog. In general, less labeled PMME and PDME were found when schistosomula were labeled with [3H]palmitic acid than when labeled with [3H]oleic acid. The labeled lipids were separated by HPLC on a 5 μm silica acid column with a gradient of hexane-isopropanol-water as described in the text and detected with a flow-through radioactivity detector; SF, solvent front; PE, phosphatidylethanolamine; PMME, phosphatidylmonomethylethanolamine; PDME, phosphatidyldimethylethanolamine; PC, phosphatidylycholine.
In general, more \[^3\text{H}\]oleic acid was incorporated into PE, PMME, and PDME than \[^3\text{H}\]palmitic acid into these same lipids. For oleic acid-labeled organisms in 200 \(\mu\)M MME, 14% of the total eluted radioactivity was found in PMME; in 200 \(\mu\)M DME, 16% of the oleic acid radioactivity was found in PDME. By comparison, for palmitic acid-labeled organism only 3% of the total was found in PMME in the presence of 200 \(\mu\)M MME and 5% in PDME in the presence of 200 \(\mu\)M DME. In media containing 200 \(\mu\)M choline as well as 200 \(\mu\)M analog, incorporation of either fatty acid into analog-containing phospholipids was decreased by approximately half compared with organisms labeled in media containing 200 \(\mu\)M analog alone (Fig. 3A and B). The presence of MME or DME did not significantly alter incorporation of palmitic acid into PE or PC. However, oleic acid incorporation into both PE and PC was inhibited by both MME and DME. Incorporation of palmitic acid into PDME reached a maximum between 20 and 100 \(\mu\)M DME (Fig. 1C). Thus, fatty acid incorporation into the analog-containing phospholipids saturated close to physiological concentrations of choline and ethanolamine (Table 1).

In summary, both palmitic acid and oleic acid are incorporated into PMME and PDME in the presence of MME and DME, respectively. No evidence of conversion of PMME to PDME was observed with either fatty acid precursor. Oleic acid was incorporated into analog-containing phospholipids at rates higher than palmitic acid was incorporated into the corresponding phospholipids. Further, the incorporation for both precursors was highest into PC, lowest into PE, and intermediate for PMME and PDME. At high concentrations of choline, incorporation of either fatty acid into PMME and PDME was diminished but not abolished, indicating competition between head group precursors.

**DISCUSSION**

These studies demonstrate that schistosomula incorporate their most abundant fatty acids preferentially into neutral lipids and PC with much less into PE. Incorporation is similar in medium modified to contain all the head group precursors in the same concentrations found in hu-

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**Fig. 3.** Distribution of labeled fatty acids among lipid classes from schistosomula cultured in various media. Schistosomula were cultured in RPMI alone or RPMI supplemented with ethanolamine, choline or choline analogs. Following a 3-h preincubation, worms were labeled for 7 h with either \[^3\text{H}\]palmitic acid (A) or \[^3\text{H}\]oleic acid (B). Labeled lipids were separated by HPLC using HPLC method II and quantitated with a flow-through radioactivity detector. Each bar represents the mean \(\pm\) standard deviation for three separate experiments. Experimental conditions were RPMI alone (solid bar), 200 \(\mu\)M ethanolamine (narrow left diagonal), 200 \(\mu\)M MME (narrow right diagonal), 200 \(\mu\)M DME (narrow cross-hatch), 200 \(\mu\)M ethanolamine + 200 \(\mu\)M choline (wide left diagonal), 200 \(\mu\)M MME + 200 \(\mu\)M choline (wide right diagonal), 200 \(\mu\)M DME + 200 \(\mu\)M choline (wide crosshatch).
worms incorporate fatty acids preferentially into PC, how either medium except for palmitic acid in modified ethanolamine (9), or glycerol, it follows that the regulation and control? There are three logical places: transport, addition of the head group (de novo synthesis or base exchange), and the addition of fatty acids by acyltransferases. Since the acyl chains are entering the phospholipid pool at a higher rate than choline, ethanolamine (9), or glycerol, it follows that the regulation is most probably due to acyltransferase activity. Much of the data suggest that there is probably a single acyltransferase with increased acylation efficiency for choline compared to ethanolamine. Further, fatty acids are incorporated into the phospholipids containing the choline analogs at relative rates intermediate to their incorporation into PC and PE. The incorporation of fatty acids into analog-containing phospholipids is also inhibited by excess choline and their incorporation reaches a maximum at a concentration similar to ethanolamine and choline. Although a head group specific acyltransferase explains these findings best, definitive proof requires isolation, localization, and characterization of the acyltransferase(s).

Several differences should be noted in results from this laboratory versus those of others. Vial et al. (17) reported that oleic acid-labeled PE is synthesized by schistosomula at a rate approximately 0.60 that of PC, while we have not found fatty acid incorporation into PE to exceed 0.16 that of PC (Table 2). Vial et al. (17) also reported that the rate of glycerol-labeled PE synthesis in schistosomula was greater than that of glycerol-labeled PC, while here the comparative rate was 55%. These differences are not attributable to exogenous ethanolamine concentration. However, their medium contained both glycerol and CoA which were lacking in both standard and modified RPMI. Addition of these components to either medium has essentially no effect on the incorporation of myristic acid or palmitic acid (data not shown). Thus the differences in relative rates of incorporation of fatty acid and glycerol precursors between laboratories are unresolved. A further difference, namely the methylation of PE to PC (4, 17) was not observed by others (18) or ourselves (9). As we have shown previously, this result is most likely due to misidentification of lyso-PE as PC (9). Additional evidence that PE methylation does not occur in schistosomula at an appreciable rate comes from the experiments with choline analogs. Although the parasite can utilize MME as a head group precursor, even at a concentration of 200 μM, further methylation of fatty acid-labeled PMME to PDME was not observed. Despite the differences noted here, there is reasonably good agreement between laboratories regarding lipid precursors that can be incorporated by the parasite and rates of synthesis of the major lipid classes.

The results of the present study are similar to work in mammalian cells. Ehrlich ascites cells (10), BHK-21 cells (11), T cells (12, 13), cultured neuroblastoma cells (14), and bone marrow-derived (15) or peritoneal macrophages (15) preferentially label PC with fatty acids compared with other phospholipid classes. These observations suggest that different phospholipid classes demonstrate different kinetic properties, with more rapid turnover of PC and slower turnover for other phospholipid classes (21). By contrast, acylation of phospholipids by protozoa appears to differ considerably from mammalian cells and from the results observed in the present study. In general, for Trichomonas (34), Plasmodium (35), and Tetrahymena (36, 37), the fatty acid precursors were not preferentially incorporated into PC but were incorporated into phospholipid classes at rates that were similar to the prevalence of the phospholipid classes. Regardless of cell type, however, it is clear that the mechanisms controlling fatty acid esterification are complex. The overall distribution of fatty acids observed among lipid classes in intact cells is the sum total of the activity of enzymes including acyltransferases, phospholipases, and fatty acid binding proteins, each of which exhibit their own substrate specificities. In S. mansoni this is further complicated by the fact that the organism contains multiple cell types. The enzymes of the synthetic pathways for phospholipids in this parasite may offer unique sites for drug action.
REFERENCES


