Methionine Methyl Group Metabolism in *Lemna*

**S. Harvey Mudd** and **Anne H. Datko**

Laboratory of General and Comparative Biochemistry, Building 32, Room 101, 9000 Rockville Pike, National Institutes of Mental Health, Bethesda, Maryland 20892

**ABSTRACT**

To provide information upon the ways in which *Lemna paucicostata* uses the methyl group of methionine, plants were grown for various periods (from 1 minute to 6.8 days) in the presence of a tracer dose of radioactive methyl-labeled methionine. Protein methionine accounted for approximately 19% of the accumulated methyl moieties; other methylated products, about 81%. The latter group included (percent of total methyl in parentheses): methylated ethanolamine derivatives (46%); methyl esters of the pellet (chiefly, or solely, pectin methyl esters) (15%); chlorophyll methyl esters (8%); unidentified neutral lipids (6%); nucleic acid derivatives (2-5%); methylated basic amino acids (2%). No other major methylated compounds were observed in any plant fraction. Available evidence suggests that little, if any, oxidation of the methyl group of methionine, directly or indirectly, occurs in *Lemna*. Our results indicate that S-methyl-methionine sulfonium is formed relatively rapidly, but does not accumulate at a commensurate rate, probably being reconverted to methionine. To our knowledge, this is the first time a reasonably complete accounting of the metabolic fate of methionine methyl has been obtained for any plant. The extent to which the results with *Lemna* may be representative of the situation for other higher plants is discussed.

In a recent study of methionine metabolism, Giovanelli *et al.* (17) administered a tracer amount of L-[^35]S, U-[^14]C]methionine continuously to *Lemna paucicostata* growing in steady state conditions. It was observed that in the protein methionine of the plants the ratio of ^14C in the methyl group to ^35S was only 20% of the corresponding ratio for the administered methionine. From this result, and the knowledge that each frond contains a total of 1.05 nmol of methionine (16), it was calculated that the methyl moiety of methionine was being formed at a rate of 5.17 nmol/frond × doubling (i.e. approximately 35 h) [9]. It was suggested that the amount not persisting as methyl moieties of methionine, i.e. 5.17 - 1.05 = 4.12 nmol/frond, was utilized for transmethylation reactions. Thus, the homocysteinyl moiety of methionine would act as a methyl carrier, and about four times as many methyl groups would be transferred to methylated products as are finally contained in methionine itself. About 57% of the putative amount of methyl groups used for transmethylation could be accounted for by formation of choline derivatives (chiefly phosphatidylcholine, with lesser amounts of phosphocholine and soluble choline) (17). In the present work, we have sought by direct determination of the metabolic fate of radioactivity originating in the methyl group of methionine to confirm the above calculations, and to identify additional methylated end-products. The results, reported here, provide a complete accounting of the ways in which *Lemna* uses methionine methyls.

**MATERIALS AND METHODS**

**Plant Material and Labeling with Radioactive Compounds.** *Lemna paucicostata* Hegelm. 6746 was grown under standard conditions in medium 4 containing 20 μM SO₄⁻² and Mes buffer (11). Labeling with radioactive compounds was carried out by growth of the plants in the presence of these compounds for the times specified in individual experiments. Unless otherwise specified, radioactive L-methionine (either ^35S, ^3H, or ^14C) was present at 4 nM; ^32PO₄⁻² at 400 μM; and ^35SO₄⁻² at 20 μM.

**Separation of Plant Homogenates into Methanol-Water-Soluble, Chloroform-Methanol-Soluble, and Methanol-Chloroform-Insoluble Pellet Fractions.** Plant samples (up to 300 fronds) were harvested, washed, and homogenized in methanol as described (17). After addition of chloroform to bring the final methanol to chloroform ratio to 2 to 1, the sample was kept in ice for at least 30 min. The methanol-chloroform-insoluble material was collected by centrifugation and washed once in methanol: chloroform (2:1). The methanol-chloroform supernatant and wash were combined and separated into methanol-water-soluble, and chloroform-methanol-soluble fractions (17). Figure 1 diagrams the sequence of separation procedures used in subsequent fractionations.

**Washing, Storage, and Aliquoting of the Pellet.** The methanol-chloroform-insoluble pellet was sequentially washed once in cold methanol to remove residual chloroform; once, or twice, in 10% TCA to remove contaminating remnants of S-methyl-methionine,² AdoMet, nucleotides, etc; and twice in 80% methanol to remove residual TCA. The TCA washes (after ether extraction) and the methanol washes were included with the methanol-water-soluble fraction described above for analysis. At this stage the pellet was resuspended in methanol for alkaline removal of methyl ester groups as described below, or for storage. Little, if any, radioactivity was released from such pellets during storage at −60°C for up to 3 months. This suspension could also be conveniently and accurately divided into small aliquots for the analyses described below. Pellets derived from nonradioactive plants were processed as described here and were used as necessary to bring each aliquot of radioactively labeled pellet to about 50 frond equivalents.

**Alkaline De-esterification of the Pellet and Determination of Alkali-Labile, Volatile Radioactivity.** Washed methanol-chloroform-insoluble pellets were used for this determination. The pellet suspension (50-300 frond equivalents) was incubated in a

1 Giovanelli *et al.* (17) expressed their results in terms of nmol/colony rather than nmol/frond. Our current preference is to express results in nmol/frond, since frond number is directly measured in each experiment. The values in nmol/frond in this paper may be compared to the results of Giovanelli *et al.* by multiplying by 4.2, the frond/colony ratio used in that report (17).

2 Abbreviations: S-methylmethionine, S-methylmethionine sulfonium salt; AdoMet, S-adenosylmethionine.
mixture of 0.12 ml methanol and 0.36 ml 0.13 N KOH at 0°C for the specified time. The reaction was stopped by neutralization by the addition of 0.09 ml 0.67 M KH$_2$PO$_4$. The material precipitating when ice-cold methanol was added to a concentration of 90% was removed by centrifugation. An aliquot of the supernatant fluid was counted directly to measure total soluble radioactivity. A second aliquot was taken to dryness in a counting vial (Buchler Vortex Evaporator, at about 30°C) for determination of nonvolatile soluble radioactivity. The difference between these samples was a measure of alkali-labile, volatile radioactivity. The methanol precipitate was used for determination of alkali-stable radioactivity by dissolving it in 0.4 ml Protosol (New England Nuclear) or 0.5 ml 70% HCOOH for counting, or was subjected to the analyses described below.

**Demethylation by Pectin Methylesterase.** Reaction conditions were similar to those described by Barrett and Northcote (1). An aliquot of washed pellet (50 frond equivalents) in an Eppendorf tube was washed twice in cold H$_2$O to remove residual methanol. The pellet was incubated, tightly stoppered, at 30°C in a reaction mixture (total volume 0.2 ml) of 25 mM K-phosphate (pH 7.5), 0.1 M NaCl, and pectin methylesterase (EC 3.1.1.11). The enzymes used were the preparation from orange peel (suspended in [NH$_4$]$_2$SO$_4$) or tomato (lyophilized powder), both obtained from Sigma. To stop the reaction the tube was cooled in ice, and methanol was added to a final concentration of 80%. Insoluble material was removed by centrifugation. Radioactivity in volatile and nonvolatile fractions of the supernatant fluid was measured as described above. The methanol precipitate was dissolved in 0.5 ml 70% HCOOH for determination of radioactivity.

**Protein Methionine Determination by Use of Cyanogen Bromide.** An aliquot of de-esterified pellet was incubated in 2-mercaptoethanol to reduce any methionine sulfoxide to methionine (16). Mercaptoethanol was removed by evaporation to dryness. The residue was dissolved in 0.85 ml 70% HCOOH containing cyanogen bromide (100 mg/ml) and incubated tightly stoppered at 23°C for 16 to 18 h (12). Radioactive methylthiocyanate was extracted into 3 ml toluene (7) (preequilibrated with 70% HCOOH). Four extractions removed essentially all of the toluene-soluble radioactivity. To measure radioactivity in unreacted material contained in the aqueous phase, it was taken to dryness and dissolved in 70% HCOOH for counting.

**Separation of DNA and RNA from Tissue Protein and Acid Hydrolysis of the Latter.** An aliquot of de-esterified pellet (200 frond eq) was washed with cold 10% TCA, then extracted with 1.3 ml 5% TCA at 90°C for 15 min (28). Insoluble material was collected by centrifugation and washed once with 5% TCA (1.3 ml). The insoluble residue was dissolved in Protosol for determination of radioactivity, or was further characterized after 6 N HCl hydrolysis (110°C for 16 h in vacuo after several flushes with N$_2$). Insoluble material remaining after HCl hydrolysis was dis-
solved in 7% HCOOH for determination of radioactivity.

Alkaline Digestion of RNA in Pellets. Aliquots of a washed, de-esterified, pellet were suspended in 1.1 ml 0.36 N NaOH at 37°C for various times. Reactions were stopped by addition of 1.3 ml 6.9% cold TCA. Insoluble material was removed by centrifugation, and washed once with 1.3 ml 5% cold TCA.

Radioactivity was determined in an aliquot of the combined supernatant and wash fluids. TCA-insoluble material was dissolved in 70% HCOOH and its radioactivity determined.

Degradation of S-Methylmethionine. Samples eluted from chromatograms were taken to dryness, dissolved in 0.5 ml potassium borate (pH 8.3), 0.053 M, and heated for various times at 100°C. The reaction mixtures were cooled, 0.05 ml 2.5 N HCOOH was added to each, and the entire solution transferred with water washes to a vial for counting radioactivity. After evaporation to dryness overnight in a stream of air at room temperature, the residues were dissolved in water and radioactivity determined.

Mild Alkaline Decylation and Acid Hydrolysis of Polar Lipid Fraction. Mild decylation was carried out with 0.2 N methanolic NaOH at room temperature essentially as described by Kates (21). For acid hydrolysis, the resulting combined methanol-water phase from each sample was evaporated to dryness and the residue was dissolved in 0.25 ml 95% ethanol to which was added 1.25 ml 1.2 N HCl. The sample was heated at 100°C for 3 h, evaporated to dryness, and the residue used for paper chromatography.

Colorimetric Determinations. Chl were extracted from fresh tissue with dimethylformamide (27) and their quantities determined spectrophotometrically, using the specific extinction coefficients of Moran (26). DNA was determined as described by Burton (5).

Paper Chromatography. Unless specified otherwise, paper chromatography was carried out on Whatman No. 1 paper, using the descending method, with development overnight (about 16 h) at room temperature. Solvents used were (all compositions specified in v/v): solvent A, 1-butanol:propionic acid:H2O (250:124:175); solvent B, 2-propanol:88% HCOOH:H2O (7:1:2); solvent C, 2-propanol:29% NH4OH:H2O (7:1:2); solvent D, phenol solution (160 g phenol and 40 ml H2O)+95% ethanol:HCl (150:40:10); solvent E, pyridine:acetic acid:NH4OH (15:9:6:1.5); solvent F, 2-propanol:88% HCOOH:H2O (24:6:6); solvent G, chloroform:methanol:acetic acid:H2O (65:35:8:4); solvent H, methanol:88% HCOOH:H2O (80:14.8:5.2).

The developed chromatograms were cut into 1 cm segments. Radioactivity on these segments could be determined directly by immersion in 3a20 counting fluid (Research Products International). Segments containing material selected for further study could then be washed with ether and stained with ninhydrin or other stains, or eluted with water to obtain the radioactive material for additional studies. In some instances, usually in order to obtain better efficiency of 14C counting, segments were eluted first individually and radioactivity determined in suitable aliquots of each eluate. The remaining portions of the eluates were then available for additional studies. Initial chromatography of chloroform-methanol-soluble fractions was carried out on Whatman 5G 81 silica gel chromatography paper, developed overnight by the ascending technique with solvent G. The developed chromatograms were cut into 1 cm segments, each of which was eluted with methanol to obtain material for determination of radioactivity and/or for additional studies.

Column Chromatography. Chromatography on small columns of Dowex 50 has been described (15).

Electrophoresis. Electrophoresis was performed as described (8). Solvents were (components specified as v/v in water): pH 1.9: 88% HCOOH, 2.5%, acetic acid, 8.7%; pH 2.9: 88% HCOOH, 1.4%, pyridine, 0.4%; pH 3.5: acetic acid, 5%, pyridine, 0.5%; pH 7.0: acetic acid, 0.31%; and gliantine, 1.0%. Electrophoretograms were processed as described for paper chromatograms.

Calculation of Results. The amount of radioactivity in a given compound was usually expressed as a percent of the total radioactivity in the plants (i.e. the sum of the radioactivities in the combined methanol-water-soluble fraction, the chloroform-methanol-soluble fraction, and the washed pellet). During subsequent chromatographic or electrophoretic treatments, the amount of the compound in question was determined as a fraction of the radioactivity recovered on that particular chromatogram or electrophoretogram. The amount relative to the original total was then calculated as the product of the fractional contributions of that compound in each of the purification steps to which it had been subjected.

Determination of Total Methylated Ethanolamine Derivatives. Lemna plants were grown to isotopic equilibrium in the presence of 32PO43-, the specific activity of which was measured using the method of Fiske and Subbarow for Pi (13). Chloroform-methanol-soluble fractions of such plants were chromatographed with solvent G, together with aliquots of the chloroform-methanol-soluble fraction of plants grown to isotopic equilibrium with L-[14C]methionine. As shown in “Results,” the latter extract provided a marker of [14C]phosphatidylcholine. The peak of 32P co-migrating with [14C]phosphatidylcholine constituted 39 to 46% of the 32P of the chloroform-methanol-soluble fraction (range in three experiments). The radioactivity of this peak was demonstrated by elution and subjecting it to mild alkaline decylation. Virtually all the 32P then distributed into the methanol-water phase (in which are found glycerylphosphoryl derivates). During chromatography with solvent H (a solvent useful for separation of such glycerylphosphoryl bases [21]) this methanol-water-soluble radioactivity co-migrated with the internal marker of [14C]glycerylphosphorylcholine. The amounts of 32P co-migrating with [14C]phosphatidylcholine in solvent G were therefore used to calculate the concentration of this substance in the plant samples. The latter values were corrected to total methylated ethanolamine derivatives by multiplication by 11.1/9.9, the factor shown by Giovannelli et al. (17) to be the ratio of total choline derivatives to phosphatidylcholine in Lemna grown under our standard conditions.

Chemicals. Chemicals were obtained from standard commercial sources. N2,N2,N2-Trimethyllysine was a kind gift from A. V. Furano, National Institute of Arthritis, Metabolism, and Digestive Diseases, Bethesda, MD.

Radioactive Compounds. Radioactive compounds were obtained as follows: L-[14C]methionine, [14C]choline, [14C]choline and L-α-phosphatidylN-methyl-[14C]choline, dipalmitoyl (Amer- sham); S-[14C]methylmethionine (ICN); [14C]AdoMet, L-[13C3]choline, [14C]choline, [14C]choline, and [14C]phosphocholine (New England Nuclear). L-[14C]Choline was purified before use by preparative chromatography with solvent B containing 10 mM 2-mercaptoethanol. S-[14C]Methylmethionine was prepared essentially as described (8) through purification of the product by chromatography on Dowex 50-NH4+. The eluted product was >98% radio-pure during paper chromatography (solvent B) or electrophoresis (pH 7.0). [3H]Phosphocholine was prepared by incubation of [3H]choline with Lemna paucicostata for 30 min, essentially as described (10). The product, contained in the methanol-water-soluble fraction of the plant homogenate, was purified by preparative paper chromatography with solvent B.

RESULTS

The experimental approach used in this work was to adminis-
methyl group, to *Lemna* plants growing under standard steady state conditions. The radioactive methionine was administered continuously for periods of either 1, 3, or 9 min, 1, 2.5, or 4 h, or 6 d or more (i.e. 4.8–5.4 doublings, or essentially to isotopic equilibrium). For the shortest time periods, L-[1-3H]methionine of high specific radioactivity was used so that measurable amounts of radioactivity would be found in products. For longer periods (1 h, or more) either L-[3H]methionine or L-[1-32P]thymidine of lower specific activity could be used. In some experiments L-[1-35S]methionine, 35SO42- or 32PO43- was administered to obtain products for comparison with those containing radioactive methyl groups. In each experiment, the volume of medium was such that plant uptake of the radioactive compound did not exceed 20% of that in the medium. Upon completion of the incubation period, plants were washed to remove external radioactive compounds, harvested by gentle filtration or aspiration of medium, and homogenized in methanol. The homogenate was fractionated to yield a washed methanol-chloroform-insoluble pellet, and components soluble in either methanol-water or chloroform-methanol (Fig. 1). In the sections which follow, we report studies the results of which identify the major radioactive compounds present in each fraction, and permit quantitative estimates of the rate of methyl flux into each major component. An outline of the procedures used to separate the various plant fractions is presented in Figure 1.

**Methanol-Water-Soluble Fraction.** After short incubation periods, the major portion of the radioactive methionine taken up as methyl-labeled methionine was found in this fraction (for example, at 1 min, 91% of total plant radioactivity). With increasing incubation times, this portion decreased (at 9 min, 74%; at 1 h, 48%; at 4 h, 28%; after 6.8 d, 13%), indicating that the major methyl-containing end-products were in the non-water-soluble fractions. The methanol-water-soluble fractions were analyzed initially by electrophoresis at pH 7.0. After all incubation times, the major methanol-water-soluble radioactive products were located in relatively few areas. These areas, and trends in the changes in quantities of radioactivity in each as a function of time of incubation, are exemplified by the representative results diagrammed in Figure 2 for incubations of 1 min, 4 h, and 6.8 d:

(a) **Methionine and AdoMet.** After short incubations (e.g. panel A, Fig. 2) most of the total plant radioactivity moved only a few centimeters toward the cathode, to the area in which authentic methionine was found. The second most prominent peak was represented by a shoulder at 5 to 10 cm toward the cathode, the area to which authentic AdoMet moved. After intermediate incubations, by which times the peak moving with methionine represented much smaller portions of the total plant radioactivity (e.g. panel B, Fig. 2), the peak moving with AdoMet clearly separated from that moving with methionine. After very long incubations, radioactivity in the AdoMet area was so small a portion of the total plant radioactivity as not to be detected on electrophoregrams of the usual sensitivity (e.g. panel C, Fig. 2). Further studies to rigorously identify the radioactive material in either the methionine or AdoMet area were not carried out during the present experiments.

(b) **Methylated Derivatives of Phosphoethanolamine.** After short incubations, the next most prominent radioactive peak in the methanol-water-soluble fractions moved 3 to 8 cm toward the anode; almost, but not exactly, as did authentic phosphocholine (panel A, Fig. 2). After intermediate incubations (e.g. panel B, Fig. 2), this peak represented a larger portion of the total plant radioactivity, and agreed in its mobility more closely with authentic phosphocholine. Even after prolonged incubation, this peak was a prominent feature of the methanol-water-soluble fraction, although it represented a lesser percent of the total plant radioactivity (panel C, Fig. 2). For each experiment this peak of radioactivity was eluted from the electrophoretogram and chromatographed on paper using solvent B. With this solvent, virtually all the radioactivity of this peak moved with authentic phosphocholine (data not shown). As will be reported in detail elsewhere, when this radioactivity was eluted and chromatographed on paper with solvent C (approximately 63 h development) three discrete peaks separated. These were identified as phosphomethylthanolamine, phosphodiethanolamine, and phosphocholine. The relative amounts of these components varied with incubation time (SH Mudd, AH Datko, unpublished observations).

(c) **S-Methylmethionine.** After short incubations (e.g. panel A, Fig. 2) a peak of radioactivity comparable in amount to that contained in the methylated derivatives of phosphoethanolamine was found 15 to 16 cm toward the cathode. This peak agreed in mobility exactly with authentic S-methylmethionine. After longer incubation periods, this peak increased as a percent of total plant radioactivity (panel B, Fig. 2), but remained at all times a well separated, readily identifiable component of the methanol-water-soluble fraction. To further characterize this material, the peaks from the pH 7.0 electrophoregrams of the 1- and 3-min incubations were eluted and chromatographed on paper with solvent B. In each case, a single peak of radioactivity was found, comigrating with authentic S-methylmethionine at 8 to 12 cm (data not shown). These areas were eluted and aliquots of each were heated for varying times at pH 8.3 and 100°C. Under these conditions S-methylmethionine decomposed to homoserine and dimethylsulfide (8). The latter volatile compound would contain any radioactivity originally present in the methyl groups of S-methylmethionine which had undergone degradation. After heating, reaction mixtures were acidified to limit further decomposition of S-methylmethionine, volatile materials were removed by evaporation, and radioactivity was determined in the nonvolatile residues. The results (Fig. 3) showed that 3H present in the putative S-[3H]methionine formed by plant metabolism became volatile at exactly the same rate as did 14C present in authentic S-[1-14C]methionine.

(d) **Choline.** The last major peak of radioactivity to appear in the methanol-water-soluble fraction was that migrating 23 to 26 cm toward the cathode. Barely detectable after 1- or 3-min incubations, this peak slowly continued to accumulate radioactivity, reaching 6 to 7% of the total plant radioactivity in plants labeled to isotopic equilibrium, and appearing to co-electrophorese with authentic choline (Fig. 2). For further study, this radioactive peak was eluted from the pH 7.0 electrophoregrams of selected experiments (the 3 and 9 min, as well as the 1, 2.5, and 4 h incubations). Paper chromatography with solvent B showed that for the sample from the 3-min incubation only 23% of the radioactivity in this area (0.08% of the total plant radioactivity) moved with authentic choline. For samples from longer incubation times, all radioactivity from this area moved as a single peak with authentic choline. After this step, all of the radioactivity in each choline area co-migrated with authentic choline during subsequent paper chromatography with solvent C.

(e) **Minor Unidentified Methylated Products in the Methanol-Water-Soluble Fraction.** To ascertain whether additional unidentified methylated end-products were present, incompletely characterized peaks of radioactivity from the electrophoregram of the 6.8 d incubation were studied further. The peak of radioactivity migrating with methionine (2–9 cm toward the cathode) was eluted and chromatographed on paper using solvent B. Most of the unidentified 14C moved with authentic methionine or methionine sulfoxide, but a discrete peak, representing 0.8% of the total plant radioactivity, was demonstrated. The peak of radioactivity at the origin of the electrophoretogram (represent-
Fig. 2. Electrophoresis at pH 7.0 of aliquots of methanol-water-soluble fractions. The times of incubation with methionine labeled with radioactivity in the methyl group were 1 min (panel A), 4 h (panel B), and 6.8 d (panel C). Electrophoresis was for 50 min. The distribution of radioactivity in each plant sample is shown by solid lines, scaled according to the left ordinates. The positions of authentic added internal radioactive markers (each named in the figure) are indicated by dashed lines, scaled according to the right ordinates. The vertical dotted line indicates the position of the origin. For panel A, the plant samples were labeled with \(^3\text{H}\); the authentic markers, with \(^1\text{C}\). For panels B and C, the plant samples were labeled with \(^1\text{C}\); the authentic markers, with \(^3\text{H}\). The percent values are the portions of the total plant radioactivity located in the bracketed areas. Note the change of scale on the left ordinate of panel A.
ing 1.0% of the total plant radioactivity ([panel C, Fig. 2]) was eluted and subjected to the same chromatographic procedure. Most of the radioactivity in this area co-migrated with authentic phosphocholine, but a small additional peak, moving more rapidly, and representing 0.3% of the total plant radioactivity, was noted. Thus, a reasonable estimate of the total unidentified methanol-water-soluble methylated products accumulating in *Lemna* during prolonged incubation would be equivalent to 0.8 + 0.3 = 1.1% of the methionine methyl moieties metabolized by the plant.

**Chloroform-Methanol-Soluble Fraction.** Radioactivity originating in methyl-labeled methionine initially appeared more slowly in the chloroform-methanol-soluble fraction than in the methanol-water-soluble, but thereafter accumulated more rapidly in the former so that, after a 2.5 h incubation, the radioactivities in these two fractions were about equal. At 4 h, and more strikingly after prolonged incubation, radioactivity in the chloroform-methanol-soluble fraction exceeded radioactivity in the methanol-water-soluble fraction. These points are illustrated in Figure 4, which depicts silica-gel paper chromatograms (solvent G) of aliquots of chloroform-methanol-soluble fractions (compare total percent radioactivities in these chromatograms with those for the methanol-water-soluble fractions derived from the same incubations, Fig. 2). Figure 4 demonstrates also that radioactivity in the chloroform-methanol-soluble fractions occurred chiefly in two areas after chromatography with solvent G:

(a) **Polar Lipid Peak.** The more slowly migrating (therefore more polar) lipid peak (Fig. 4) became labeled with radioactivity only after an appreciable lag, but thereafter accumulated radioactivity rapidly (panel B, Fig. 4), and became the quantitatively dominant product (35% of total plant radioactivity) after labeling to isotopic equilibrium (panel C, Fig. 4).

The chromatographic mobility of this peak was not significantly different from that of authentic phosphatidylcholine. To further characterize the metabolic products(s) in this area, after elution each sample was subjected to mild alkaline deacylation in methanol (21). This procedure removes acyl groups from glycerol-phosphatides, yielding methyl esters of the fatty acids and glycerylphosphoryl derivatives. These products are separated by distribution of the fatty acid methyl esters into chloroform, while glycerylphosphoryl derivatives are found in the methanol-water phase. Acid hydrolysis of glycerylphosphoryl bases yields the free bases. After incubation times of less than 9 min, most of the radioactivity migrating to the area of phosphatidylcholine was found in the fatty acid methyl ester fraction after mild alkaline deacylation. With samples derived from longer incubations, the portion of radioactivity behaving as glycerylphosphoryl base increased, rising to 81% by 1 h and 97% in the equilibrium-labeled sample. After acid hydrolysis, essentially all the radioac-

---

**Fig. 3.** Characterization of S-methylmethionine. 3H-Containing samples had been formed by the plants during incubation with L-[3H]methionine, for either 1 min (△, ▲) or 3 min (○, ●). These samples had been copurified with authentic L-[3H]S-methylmethionine as described in the test. Samples were heated at 100°C at pH 8.3 for the times shown, and remaining nonvolatile radioactivity determined. (△, ○), 3H; (▲, ●), 14C.

**Fig. 4.** Silica gel paper chromatography of aliquots of chloroform-methanol-soluble fractions. Times of incubation and isotopes utilized were as in Figure 1. The cross-hatched area marked YG represents the area near the solvent front to which the yellow-green visible plant pigment migrated. The percent values are the portions of the total plant radioactivity.
tivity in the glycerolphosphoryl base fractions derived from incubations of 9 min, or longer, co-migrated with authentic choline during paper chromatography with solvent B. Thus, for example, in the equilibrium-labeled sample the methyl groups of choline accounted for $35 \times 0.97 = 34\%$ of the total radioactivity in the plants.

(b) Nonpolar Lipid Peak. The more rapidly migrating (therefore 'nonpolar') lipid peak (Fig. 4) contained most of the radioactivity of the chloroform-methanol-soluble fraction after short incubations (panel A). With longer incubations, radioactivity continued to accumulate in this peak, albeit at a slower rate than did radioactivity in polar lipid. After 4 h, the relative contributions of these two peaks changed relatively little (panels B and C). Since this peak moved to the same area of the chromatogram as did Chl (Fig. 4), it was considered that at least a portion of the radioactivity in question might be in the form of Chl methyl esters. To explore this possibility, samples of material from this peak were treated with methanolic NaOH. The reactions were stopped by acidification with acetic acid, the products were distributed between methanol-water and chloroform, and the methanol-water-soluble material was examined for volatile radioactivity. After incubation in a mixture of 1 ml methanol and 0.8 ml 1.5 N NaOH at 23°C for 16.4 h, 56\% of the radioactivity originally in the nonpolar lipid peak was found in the methanol-water phase in a volatile form. The remaining radioactivity continued to distribute into the chloroform phase after this NaOH treatment.

To prove more definitively that this volatile radioactivity originated in Chl, an aliquot of a chloroform-methanol-soluble fraction from plants incubated for 4.8 doublings with L-$[^3H]$C]methionine was chromatographed under conditions in which Chl move slightly ahead of phosphatidylcholine, and behind a rapidly migrating peak of radioactivity thought to consist of neutral lipids (Fig. 5). The eluates comprising each $^3$H-containing peak were combined and treated with NaOH at 23°C for 16.4 h (conditions as above). The portions of the radioactivity in each peak which became volatile were as follows: peak at $-1$ to 3.5 cm, 8\%; peak at 3.5 to 8 cm, 95\%; peak at 8 to 14 cm, 96\%; peak at 23 to 28 cm, 14\%. Thus, the product peaks in which the radioactivity was converted by base treatment to a volatile form were those migrating with Chl. These results are in agreement with the observation that 56\% of the total radioactivity of the nonpolar lipid peak after chromatography with solvent G became volatile during base treatment. This nonpolar peak should contain both Chl and neutral lipids, and, from the results illustrated in Figure 5, should thus yield approximately $(2.3 + 2.4)/(2.3+2.4+3.5) \times 100\% = 57\%$ volatile radioactivity after base treatment.

**Washed Methanol-Chloroform-Insoluble Pellet.** Radioactive methyl-containing compounds expected to be found in this washed pellet include protein methionine, methylated amino acids of proteins, methylated bases and sugars contained in nucleic acids, and the methyl ester groups of pectin.

(a) Methyl Esters. To search for methyl esters, portions of the washed pellet were treated with KOH in aqueous methanol and the reactions were stopped at various times by neutralization. After removal of methanol-insoluble material, radioactivity was determined in portions of the supernatant fluids, either with or without evaporation to dryness. Exposure to 0.1 N KOH at 0°C resulted in very rapid (half-time about 1 min) conversion of a portion of the radioactivity in the washed pellet to a methanol-soluble, volatile form (Fig. 6, panel A). This reaction attained completion in 15 to 30 min, with conversion of approximately 40\% of the radioactivity in a pellet derived from plants grown for 4.8 doublings with methyl-labeled methionine. In this particular experiment the washed pellet contained 26.5\% of the total plant radioactivity. The methyl esters of the pellet therefore contained 10.06\% of the total. Treatment with 1 N KOH at 2°C for 15 h yielded the same proportion of volatile radioactivity (data not shown). Treatment with either orange peel or tomato pectin methylsterase also converted the same portion of radioactivity to a methanol-soluble, volatile form (data not shown). In each instance the reaction was a little more than 65\% complete in 15 min at 30°C in the presence of 0.05 units of enzyme (0.7 $\mu$g of the orange peel preparation; 0.3 $\mu$g of the tomato) and virtually complete with 0.5 to 1 unit. Based on these results, in most experiments the contribution of methyl esters to the total radioactivity of the washed pellet was determined as the percent converted to a methanol-soluble, volatile form by a 15 to 30 min incubation in 0.1 N KOH at 0°C. This treatment was also used to de-esterify pellets which were to be further studied for protein methionine, methylated amino acids, or methylated bases and sugars of nucleic acids.

(b) Methyl Group of Protein Methionine. To determine the contribution of the radioactive methyl group of protein methionine, de-esterified pellets were treated with cyanogen bromide under conditions in which the thiomethyl moieties of protein methionine are efficiently converted to methylthioisocyanate (12). The latter was extracted into toluene (7) and its radioactivity determined. In duplicate assays of a pellet derived from plants labeled for 4.8 doublings with L-$[^3H]$C]methionine, 72.8 and 73.0\% of the radioactivity initially present in the de-esterified pellet was converted to a toluene-extractable form. The reaction was completely dependent upon the presence of cyanogen bromide. A control experiment was performed with a similar pellet derived from plants incubated for 1.3 doublings with 40 $\mu$M L-$[^3S]$methionine. In such a pellet virtually all the radioactivity is expected to be in the thiomethyl moiety of $[^3S]$protein methio-
nine. Duplicate assays yielded 91.7 and 91.7% of cyanogen bromide-dependent, tolune-extractable radioactivity. It was concluded that in the de-esterified pellet of the plants labeled with L-[3H]C methionine at least 72.9% of the radioactivity was present as the methyl group of protein methionine; possibly as much as 72.9/0.917 = 79.5% was in this form. Since in this particular experiment the de-esterified pellet contained 15.9% of the total plant radioactivity, the portion of the total in the form of methyl moieties of methionine was 11.6 to 12.6%.

For further studies, de-esterified pellets were extracted with 5% TCA at 90°C for 15 min, a procedure which splits both DNA and RNA from proteins, leaving the latter as a TCA-insoluble residue (28). This treatment solubilized 21.1 and 20.0% of the total radioactivity in pellets derived, respectively, from plants grown for 4.8 doublings in the presence of L-[3H]C methionine or L-[4CH3]C methionine. (For subsequent studies of this fraction see section (d), below, on 'methyl groups in nucleic acids'.) Similar treatment solubilized 92% of the radioactivity of a pellet from plants grown for 5.7 doublings in 33P-O4−, but only 1% of the radioactivity of a pellet from plants grown for 5.3 doublings in 35SO4−. The TCA-insoluble residues from the pellets grown with either L-[3H]C methionine or L-[4CH3]C methionine were then hydrolyzed in 6 N HCl. Virtually all (99%) of the remaining radioactivity was solubilized. The soluble material was passed through a column of Dowex 50-NH4+. The retained material, eluted with 3 N NH4OH, contained 6 to 7% of the total radioactivity added to the column. (For subsequent studies of this fraction see section [c], below, on 'methylated basic amino acids'). The combined flow-through and water washes contained 93 to 94% of the added radioactivity. Chromatography of aliquots of the latter fractions (solvent A) revealed only two peaks of radioactivity, approximately 82% moving with authentic methionine, and 18% moving in the area to which both methionine sulfoxide and methione sulfone traveled. Together, these results provide further evidence that approximately 80% × 0.93 = 74% of the radioactivity in the de-esterified pellets was in the form of protein methionine, and that maxima of approximately 6 and 20% could be, respectively, in the form of N-methylated basic amino acids or methylated bases and sugars in nucleic acids. These values represent 11.8, 1.0, and 3.2% of the total plant radioactivity.

(c) Methylated Basic Amino Acids. The fraction of the 6 N HCl hydrolysate retained by Dowex 50-NH4+ was further investigated for N-methyl derivatives of arginine and lysine. During chromatography with solvent E (Fig. 7) radioactivity in this fraction of the plant sample separated into three major peaks (1, 2, and 3 of Fig. 7). A tentative designation of which methylated derivative(s) of lysine or arginine might be present in each of these peaks was made by comparison of their relative mobilities with those of authentic compounds, (when available), or with the mobilities reported by Klagsbrun and Furano (22). The relative mobilities of authentic compounds observed by these workers during two-dimensional chromatography on thin layer cellulose sheets agreed closely with those we measured during the present studies using paper chromatography. Each unknown peak of radioactivity was eluted and rechromatographed with solvent F, together with appropriate authentic compounds for ninhydrin staining. Peak 1 was identified as N2,N2,N3,N3-dimethyllysine, the only methylated lysine or arginine derivative to move clearly ahead of methionine sulfoxide during chromatography with solvent E. Radioactivity of this peak also co-migrated with authentic N2,N3,N3-dimethyllysine with the expected mobility during chromatography with solvent F. Peak 3 was tentatively identified as N2,N2,N3-trimethyllysine because of its slow mobility with solvent E. With solvent F, 55% of the radioactivity of this peak co-migrated with authentic N2,N3,N3-trimethyllysine to the expected position just in front of methionine sulfoxide. Peak 2 was assumed by virtue of its mobility in solvent E, and by exclusion, to be either N2-methyllysine or a methylated arginine. With solvent F, the radioactivity of this peak moved slightly ahead of authentic N2-methyllysine, and was therefore taken to be a methylated arginine, although appropriate authentic markers were not available.

(d) Methyl Groups in Nucleic Acids. The material extracted from the de-esterified pellet by 5% TCA at 90°C was further examined for methylated bases and sugars of nucleic acids. After either extraction to remove TCA, the extract was hydrolyzed in 1 N HCl at 100°C for 1 h to form from nucleic acids free purine bases and pyrimidine nucleotides (23). The hydrolysate was chromatographed on Dowex 50-H+, yielding a combined flow-through and water wash, and a retentate eluted with 2 N HCl. The former fraction contained about 90% of the radioactivity recovered from the column; the retentate, about 10%. During electrophoresis of the retentate at pH 3.5 all the plant radioactivity in this fraction behaved similarly to purine bases, moving toward the cathode, and forming a broad area of poorly resolved peaks near those of authentic radioactive guanine and adenosine. During electrophoresis of the combined flow-through and water wash at pH 7.0, 75% of the radioactivity in this fraction behaved similarly to nucleotides, moving toward the anode and forming several poorly resolved peaks near those of authentic AMP or UMP. Although individual components of these fractions were not further identified, these results suggest that, of the total radioactivity in the hot TCA extract, at least 10% + (0.75 × 90%) = 77.5% was in the form of methylated derivatives in nucleic acids. The contribution of these compounds to total plant radioactivity would then be 0.775 × 3.2% = 2.5%.

To gain further information upon the extent to which methyl
groups originating in methionine might be expected to accumulate in nucleic acid derivatives, the following procedure was used: Washed pellets were prepared from three plant samples grown to isotopic equilibrium in medium containing $^{32}$P of known specific activity. The mean $^{32}$P content of the pellets, 2.31 ± 0.12 (SE) ng atom/frond, provided a measure of total nucleic acid phosphorus. The contribution of RNA to total nucleic acids was measured by determining the fraction of $^{32}$P in these pellets which became TCA-soluble during treatment with base. During 1 h in 0.36 N NaOH at 37°C, 87% of the total $^{32}$P of such a pellet was converted from a TCA-insoluble to a TCA-soluble form. Analogous treatment of a pellet from plants grown to isotopic equilibrium in $^{32}$SO$_4^{2-}$ converted less than 2% of the total $^{32}$S. The remaining $^{32}$P was resistant to solubilization (for example, 90% of the total $^{32}$P converted during a 24 h incubation). These results suggest that approximately 90% of the total plant nucleic acid nucleotides are in RNA. In agreement, DNA determination by the diphenylamine reaction in two samples of control plants gave a value equivalent to 0.20 ± 0.01 ng atom DNA P/frond, or 0.20/2.31 × 100% = 8.7% of the total plant nucleic acid phosphorus. Using these values, assuming that ribosomal RNA accounts for about 70% of the total cellular RNA, and transfer RNA about 25% (18), and utilizing the values summarized by Hall (19) for the occurrence in plants of modified ribonucleosides and deoxyribonucleosides, it was estimated that the 2.31 neq of nucleoside/frond might reasonably be expected to contain 0.12 neq methyl moieties originating in methionine. In Table I (see “Discussion”) this estimate is compared with that derived from the rate at which radioactivity from methyl-labeled methionine accumulated in nucleic acid derivatives.

Lack of Additional Methyl-Containing Products. Efforts were made to detect certain other products which might have contained some of the metabolized methyl moieties of methionine.

(a) Serine. The 6 N HCl hydrolysate of a de-esterified, hot TCA extracted pellet derived from plants grown for 4.8 doublings with L-$[^{14}$C]methionine was used to search for $[^{14}$C]serine. An aliquot of this hydrolysate to which had been added an internal marker of authentic $[^{14}$C]serine was chromatographed sequentially with solvents A and D. All $^{14}$C was separated from the $[^{14}$C]serine. The sensitivity of this experiment was sufficient to detect $[^{14}$C]protein serine had it contained as much as 0.01% of the total plant radioactivity.

(b) 1-Methylhistidine or 3-Methylhistidine. No detectable amount of either of these compounds labeled with $^{14}$C was found in the protein hydrolysate from the plants labeled to isotopic equilibrium with $[^{14}$CH$_3$]methionine.

(c) Volatile Compounds. Volatile metabolites derived from the methyl moiety of methionine (other than the methyl esters of the pellet and Chl already discussed) were searched for by determination of radioactivity, with and without drying, of aliquots of the methanol-water-soluble fractions of plants following incubation with methyl-labeled methionine. No volatile material was detected. Similarly negative results were obtained by analogous studies of the media in which the plants had been incubated. During some experiments, air was bubbled through the growth media and the gas phases above, and passed through traps of either methanol at dry-ice temperature or ethanolamine in methylcellosolve (3). No radioactivity was detected in these traps. Plants which had been incubated with methyl-labeled methionine and immediately homogenized in a mixture of 0.9 ml 1 N KOH and 2.1 ml methanol contained the expected amount of volatile radioactivity. To search for acidic, volatile radioactive compounds, further aliquots of these homogenates were acidified and counted with and without drying. No additional increment of volatile radioactivity was obtained. Together, these results fail to provide any evidence that a significant fraction of the methyl groups of methionine are metabolized by Lemna to either CO$_2$, formate, formaldehyde, or methanol (other than that derived from methyl esters).

Quantitation of Fluxes. In the results presented to this point, the amounts of radioactivity in specific compounds are expressed in terms of percent of total plant radioactivity. To permit conversion of these values into actual fluxes, the data were replotted as in Figure 8, in which the amount of radioactivity in a given compound (or group of compounds) after a specified period of incubation is expressed relative to the radioactivity in the de-esterified pellet from the same incubation. These results are interpreted in the “Discussion.”

DISCUSSION

In the present work we have attempted to provide a reasonably complete accounting of the metabolic utilization of methionine methyl groups by Lemna paucicostata growing under standard conditions. Each Lemna frond contains 1.05 nmol protein methionine, but additional moieties derived from methionine methyls accumulate in a variety of materials. Table I summarizes these results in quantitative terms. To arrive at the calculated values for methyl equivalents listed in this Table, the data of Figure 8 were used. As this Figure shows, incorporation of radioactivity into total methylated ethanolamine derivatives, into methyl esters of the pellet, and into neutral lipid each shows a lag relative to incorporation into the de-esterified pellet. These lags are presumed to be due to the fact that radioactive methionine must pass through a pool of AdoMet before entering the methylated compounds in question, whereas it enters protein...
without passage through this pool. During the later times of Figure 8 the relative radioactivity in each of these fractions changed little, indicating saturation of precursor pools, and making it possible to calculate the accumulation of each compound, based on the known accumulation of protein methionine.3 For example, in Figure 8 the final ratio of radioactivity in total methylated ethanolamine derivatives to that in the de-esterified pellet is 1.71. The de-esterified pellet is known to contain 1.05 nmol/frond protein methionine (16) which contributes 74% of the radioactivity in this pellet. Therefore, the total methyl moieties in methylated ethanolamine derivatives is calculated to be: (1.71/[1.00 x 0.74]) x 1.05 = 2.43 nmol/frond. It is noteworthy that there is reasonably good agreement between the values calculated in this way for accumulation of radioactive methionine methyls in either total methylated ethanolamine derivatives or in Chl methyl esters, and the amounts of each of these classes of compounds estimated to be present on the basis of independent measurements. These results suggest that the values for those fractions of Table I determined solely by accumulation of radioactive methionine methyls (methyl esters of the washed pellet; neutral lipids; and methylated basic amino acids) are likely also to be reasonably reliable.

The actual fluxes of methyl groups into the compounds of Table I would be higher than the values for accumulation to the extent that there is turnover of the methyl moieties in question. Such turnover or degradation should manifest itself in Figure 8 by a decrease with time in the ratio of radioactivity in any compound from which methyl groups are removed and replaced, relative to the radioactivity of a compound in which methyls continue to accumulate without removal. In Figure 8, 'methylated ethanolamine derivatives' furnish a reference group of the latter type. Giovannelli et al. (17) have shown that in Lemna growing under our standard conditions there is no significant degradation of the methyl groups of choline or choline derivatives. After about 240 min incubation no decrease is observed in Figure 8 in the radioactivity of the de-esterified pellet, in radioactive methyl esters of the washed pellet, or in radioactivity in neutral lipid, each taken relative to radioactivity in methylated ethanolamine derivatives. These results suggest that any turnover of the methyl-derived moieties of the specified fractions is minor relative to their rates of accumulation. This lack of any indication of significant turnover is supported by our failure to find radioactivity derived from methionine methyls in compounds such as CO₂, HCOOH, HCHO, methanol (other than that liberated from the methyl esters of the pellet or Chl), or in major amounts in any unidentified compound in any fraction of the plants. Any turnover of the compounds of Table I requiring replacement of methyl moieties should be balanced by the appearance of radioactivity in other end-products. In this regard, the failure to find any 14C derived from L-[4-14C]methionine in protein serine is particularly noteworthy, providing evidence that [14C]formaldehyde is not formed in significant quantities by Lemna from L-[4-14C]methionine; either indirectly by degradation of any meth-
ylated compound, or directly by an oxidative reaction affecting the methyl moiety of methionine itself.

Treating the values of Table I as fluxes, the total calculated utilization of methionine methyl moieties is seen to be 5.49 neq/fenor, in excellent agreement, when account is taken of experimental uncertainties, with the value of 5.17 reported by Giovanelli et al. (17) on the basis of entirely different methodology. The amount utilized in formation of compounds other than methionine is then 5.49 - 1.05 = 4.44 neq/fenor. Of the 4.44 neq, 2.43 (or 55%) goes toward methylation of ethanolamine derivatives, confirming the preeminent role of this pathway in the utilization of methionine methyls (17). The major amounts of methyl moieties in this group occur in phosphatidylethanolamine, soluble choline, and phosphocholine, in that order (17), but also included are what will be shown elsewhere to be small amounts of phosphomethylglycerolphosphate and phosphomethylsphinganine. Although minor in amount, these compounds are important because they turn over rapidly and serve as the major, perhaps sole, conduit for entry of methionine methyls into this group. In support of the latter possibility, we have recently demonstrated that growth of Lemna in the presence of exogenous choline brings about a marked down-regulation of the in vivo flux of methionine methyls into ethanolamine derivatives, producing a major reduction in the overall utilization of methionine methyls (SH Mudd, AH Datko, unpublished observations). Of additional interest is our finding that growth of Lemna in the presence of exogenous choline brings about a marked down-regulation of the in vivo flux of methionine methyls into ethanolamine derivatives, producing a major reduction in the overall utilization of methionine methyls (SH Mudd, AH Datko, unpublished observations). As previously pointed out (17), phosphatidylethanolamine is the dominant phospholipid in most plants (14); the major role of this compound as an end-product of methionine metabolism is unlikely to be limited to Lemna.

Quantitatively, the second most important end-product listed in Table I is methyl esters of the pellet, accounting for (0.85/4.44) × 100% = 19% of the methyl moieties not accumulating in methionine itself. The bond which attaches this material to the insoluble pellet seems roughly comparable in its alkaline sensitivity to that of pectin methyl esters (2, 20). In our experiments virtually the same amounts of volatile radioactivity as could be liberated by alkali were released by small quantities of pectin methyl esterase from either of two different sources (Fig. 6). Since this enzyme is highly selective for methylpolyplyphospho-uronates (24), the latter finding provides strong evidence that the major portion, perhaps all, of the methyl esters of the pellet are indeed esters of pectin. The possibility has not been eliminated, however, that other methyl esters (for example, methyl esters of protein aspartate) may make minor contributions to this fraction. Hart and Kindel (20) found that 1.0 to 3.5% of the total cell wall D-galacturonic acid residues of Lemna minor were esterified, a percent of esterification these authors judged to be low. Lemna is thus not usually high in pectin methyl ester content; in other plants, also, formation of pectin methyl esters is likely to be a major consumer of methionine methyls.

Formation of Chl methyl esters required 0.45 to 0.53 neq equivent methyl moieties/fenor, or 10 to 12% of the methyl moieties not accumulating in methionine. At a mean fodor weight of 509 µg (9), this is equivalent to 0.88 to 1.04 µmol Chl/g fresh weight of Lemna. These plants had been grown axiothermically in dim light, and were clearly less green than if they had grown photoautotrophically at high light intensity (11). Among the 14 species of sun or shade plants reviewed by Boardman (Table I of [4]) Chl content ranged from 1.78 to 3.78 µmol/g fresh weight of leaf. Thus, it seems likely that most green plant tissues will utilize somewhat more methionine methyls to form Chl methyl esters than the value reported in Table I.

Material derived from methionine methyls and accumulating in neutral lipids accounted for 8% of the moieties not ending in methionine. Marshall and Kates (25) observed similar material after incubation of spinach leaves, or slices of such leaves, with L-[14C]methionine, but did not quantitate the flux into this material relative to total methionine methyl utilization. These authors suggested the possible incorporation of methionine methyls into cyclopropane fatty acids. However, neither their work, nor our present studies, have definitively identified the neutral lipids into which both hydrogen and carbon originating in the methyl group of methionine are incorporated. Until more information is available it is not possible to arrive at even a tentative opinion as to how important this route for methionine methyl utilization is in plants in general, or to be certain whether the radioactivity originating in methionine methyls is transferred into these compounds by an AdoMet-dependent transmethylation reaction (as is likely for the other compounds listed in Table I), or by some other sort of reaction.

Finally, the values of Table I clearly demonstrate that transmethylations to nucleic acids or to proteins are likely to represent only minor pathways from the point-of-view of the quantity of methionine methyls utilized. The former pathway accounted for 3 to 6% of the moieties not accumulated in methionine; the latter pathway, 2%. There is no reason to think that these results are not representative for other rapidly growing plant tissues.

S-Methylmethionine provides a striking exception to the general lack of turnover of methylated products. The results of Figure 8 clearly show that the early rate of incorporation of methionine methyl into this compound is rapid, approaching the rate of incorporation of methyls into total methylated ethanolamine derivatives. However, as incubation times increased, the radioactive methyl moieties in S-methylmethionine decreased markedly relative to the amounts in the de-esterified pellet, or to the amounts in the other methionine methyl derived products. Metabolic reactions in which S-methylmethionine is known to participate in higher plants suggest the possible operation of a cycle:

1. ATP + methionine → AdoMet + PPI + Pi
2. AdoMet + methionine → S-methylmethionine + S-adenosylhomocysteine
3. S-Adenosylhomocysteine → homocysteine + adenosine
4. S-Methylmethionione + homocysteine → 2 methionine

Sum: ATP → Adenosine + PPI + Pi

This cycle would consume AdoMet, and explain the entry of methionine methyls into S-methylmethionine, and their exit therefrom. However, such a cycle would remain cryptic in the sense that it results in neither net consumption of methionine or its methyl group, nor net accumulation of S-methylmethionine or any other methylated endproduct. Thus, it would not contribute to the total net methionine methyl utilization of Table I. We have recently demonstrated in crude, cell-free extracts of Lemna enzymes which catalyze reactions (2) and (4) (SH Mudd, AH Datko, unpublished observations). Our present results thus provide strong evidence for the relatively rapid operation of this cycle in Lemna.

Whether the pattern of utilization of methionine methyl groups demonstrated in this paper for Lemna applies to other plant tissues is not established with certainty because, to our knowledge, comparable comprehensive studies of methionine methyl metabolism in other plants have not been reported. As noted in the foregoing discussion of the individual methylated compounds, there are sound reasons for thinking that Lemna may be representative of rapidly growing, green plant tissues in its requirement for phosphatidylethanolamine, pectin methyl esters, Chl methyl esters, and methylated amino acids and nucleic acid
derivatives, and that the present results are therefore of general application within the plant kingdom.4

LITERATURE CITED


4 Splitstoesser and Mazelis (29) administered L-[14CH3]methionine to germinating seedlings of three families of dicotyledons (1000 nmol given to unspecified weights of shoots and attached leaves, largely over a 3 h period, with incubation for up to a total of 24 h). Products formed from methionine methyl included methionine sulfoxide, S-methylmethionine, serine, "lipid" (not further identified), glucose, fructose, sucrose, additional unidentified compounds of the water-soluble neutral and acidic fractions, CO2 (up to 4% of the administered radioactivity in 24 h), and ethanol-insoluble materials. Numerous differences in experimental design, analytical methods, and mode of expression of results preclude detailed comparisons between the observations of these workers and our own. Among the important differences are: (a) uncertainty as to whether the amount of methionine administered by Splitstoesser and Mazelis constituted a tracer dose, or was enough to change pool sizes and metabolic fluxes significantly; and (b) lack of knowledge of the growth rate of the seedlings under the conditions employed. A striking difference is the formation of radioactive serine and CO2 by the seedlings, whereas we did not detect measurable radioactivity in either protein serine or in CO2. Since the seedlings were apparently not axenic, a contribution of microorganisms to the formation of these compounds cannot presently be ruled out. Although there may be real differences in the patterns of methionine methyl utilization by the dicotyledonous seedlings in question and the monocot. Lemma, in our opinion it is premature to regard this conclusion as established until the experimental differences mentioned above have been investigated.

Anal Biochem 61: 243-247
16. Giovaneli J, SH Mudd, AH Datko 1981 Recycling of methionine sulfur in a higher plant by two pathways characterized by either loss or retention of the 4-carbon moiety. Biochem Biophys Res Commun 100: 831-839
24. Macdonnell LR, R Jang, EF Jansen, H Lineeweaver 1930 The specificity of pectinesterases from several sources with some notes on purification of orange pectinesterase. Arch Biochem 28: 260-273

Copyright © 1986 American Society of Plant Biologists. All rights reserved.