Pathways of Sphingomyelin Metabolism in Cultured Fibroblasts from Normal and Sphingomyelin Lipidosis Subjects*

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The metabolism of endogenous sphingomyelin labeled with $^{32}$P or [methyl-$^3$H]choline and of exogenous [choline-methyl-$^3$H], [32P$^-$], or [N-acyl-1-$^4$C]sphingomyelin was studied in normal and Niemann-Pick Type A (NP-A) cultured fibroblasts. Despite a >96% decrease in lysosomal sphingomyelin activity in the NP-A cells, they were able to degrade endogenously produced [32P$^-$] or [methyl-$^3$H]sphingomyelin at normal or near normal rates. Exogenous [methyl-$^3$H], [methyl-$^3$H, $^{32}$P$^-$], and [methyl-$^3$H, N-acyl-1-$^4$C] sphingomyelin was taken up intact by normal and NP-A cells, with NP-A cells accumulating 4-8 times more lipid. By 20 h, 50% of the control cell-associated $^3$H and $^{32}$P was recovered in lecithin, and the ratio of activities ($^3$H/$^{32}$P) indicated most of the phosphorycholine derived from sphingomyelin had been transferred intact. By comparison in NP-A cells, after a 40-h incubation only 20% of the labeled phosphorycholine derived from sphingomyelin was recovered in lecithin. With both cell lines, 20 to 50 times more sphingomyelin was hydrolyzed than was taken up by the cells; the reaction products in the medium were ceramide and a mixture of water-soluble compounds such as phosphorylcholine and choline. These results indicate that there are at least two metabolic pathways for sphingomyelin modification in cultured fibroblasts in addition to degradation by the lysosomal acid sphingomyelinase. One route is hydrolysis by a cellular sphingomyelinase. The second is the hydrolysis and/or transfer of phosphorylcholine from sphingomyelin and results in the synthesis of lecithin.

At least three sphingomyelinases (sphingomyelin phosphodiesterase, EC 3.1.4.12) have been described in mammalian tissues. One is lysosomal, has an acid pH optimum, and is widely distributed in mammalian tissues (1, 2). Deficiency of this enzyme is part of the biochemical phenotype of some forms of the inherited sphingomyelin lipidosis called Niemann-Pick disease (2-6). A second sphingomyelinase described by Rao and Spence (7) and by Gatt (8) has a neutral pH optimum, is stimulated by Mg$^{2+}$ ions, and is particularly enriched in certain neuronal areas in the central nervous system (9). Its activity is very low in non-neural organs including cultured fibroblasts (9) but is normal in neural tissues from subjects affected with Niemann-Pick disease (6).

A third sphingomyelinase, with a neutral pH optimum and no Mg$^{2+}$ requirement, has been described in rat brain myelin (10).

In an attempt to identify the primary defect in those variants of Niemann-Pick disease in which there is sphingomyelin storage despite apparently normal lysosomal sphingomyelinase activity (11), we turned to a study of the metabolism of phospholipids in general, and of sphingomyelin in particular, in cultured fibroblasts. As a first step, we studied the metabolism of endogenous and of exogenously added phospholipids by normal and NP-A fibroblasts in order to develop experimental conditions that can clearly delineate the mutant phenotype in culture. These studies, in addition to demonstrating the storage of sphingomyelin by the mutant cell lines, have shown that these cells can degrade substantial amounts of sphingomyelin. On the basis of these studies we are suggesting that there are at least three separate pathways for sphingomyelin degradation in mammalian tissues.

EXPERIMENTAL PROCEDURES

Materials

Minimal essential medium (Eagle's), penicillin, and streptomycin, fetal calf serum, and trypsin were obtained from Gibco Canada Ltd., Burlington, ON, [H]$^3$H methyl iodide (20 mCi/mmol), [methyl-$^3$H]choline chloride (70 Ci/mmol), H$_2$$^{32}$PO$_4$, and [methyl-$^3$H]thymidine (6 Ci/mmol) were obtained from New England Nuclear Canada, Lachine, PQ. Other reagents were from various suppliers as follows: thiopehnel (Aldrich); sphingomyelin, bovine serum albumin, and phospholipase C (Sigma); Beckman Ready-solvHP scintillation fluid (Beckman Instruments); Fluorescamine (Pierce Chemical Co.); and Silica Gel HF-precoated plates, 250 $\mu$m thick (Mandel; Scientific Co., Rockwood, ON). Other chemicals and supplies were obtained from Fisher.

Control fibroblasts were cultured from biopsies from the forearm of normal volunteers or from human foreskin. Cells from patients with Niemann-Pick disease Type A were obtained from the Human Genetic Mutant Repository, Camden, NJ (Line GM-112) and from Dr. J. A. Lowden, Hospital for Sick Children, Toronto, ON (Line 318).

Methods

Synthesis of Radiolabeled Substrates—[methyl-$^3$H]Sphingomyelin (41.5 mCi/$\mu$mol) was synthesized from bovine brain sphingomyelin and [H]$^3$H methyl iodide (12). [N-palmitoyl-1-$^1$C]Sphingomyelin (82 mCi/$\mu$mol) was synthesized from sphingosylphosphorylcholine (prepared from beef brain sphingomyelin; Ref. 13) and [1-$^1$C]palmitic acid (14, 15). [methyl-$^3$H, $^{32}$P]Sphingomyelin was prepared by incubating a confluent cell sheet of normal cells in a roller bottle (700 cm$^2$) with 30 ml of medium containing 0.8 mCi of [methyl-$^3$H]choline and 5 mCi of $^{32}$P for 96-120 h. The cell lipid fraction was isolated, and the [methyl-$^3$H, $^{32}$P]sphingomyelin was isolated and characterized as described later (see "Harvesting of Cells"). [methyl-$^3$H, $^{32}$P]Phosphorylcholine was prepared from the labeled lecithin generated during

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† Career Investigator of the Medical Research Council of Canada.

$^1$ The abbreviation used is: NP-A, Niemann-Pick Type A.
Sphingomyelin Metabolism

the biosynthesis of the [methyl-H, 32P]sphingomyelin. The lecithin was incubated with phospholipase C (16), and the phosphocholine released was purified by preparative TLC in solvent C (7).

The sphingomyelin substrates were purified and chromatographed with authentic sphingomyelin in four solvent systems (7). On digestion with phospholipase C (16), >99% of the radioactivity of N- [methyl-3H]sphingomyelin was recovered in the corresponding solvent C (7). For the [methyl-H] and [methyl-H, 32P]sphingomyelin, >98% of the product of phospholipase C digestion was water-soluble and chromatographed as phosphocholine or choline on thin layers of cellulose C (New England Nuclear Canada) developed in n-propyl alcohol, 90% formic acid, H2O (60:24:16) (all solvents by volume) or thin layers of cellulose G developed in methanol, H2O, 30% NH4OH (69:35:5). (While the product of phospholipase C digestion should be phosphocholine, we have also observed some choline formation with authentic standards, suggesting the presence of phosphomonoesterase in the phosphatidylcholine C preparation.)

Cell Culture— Fibroblasts were grown in Eagle’s minimal essential medium containing 10% fetal bovine serum. Penicillin (100 units/ml) and streptomycin (100 μg/ml) were added only during lipid uptake experiments. Cells were between the 3rd and 14th passage when used and had been subcultured into 60-mm plastic dishes (Falcon) or 700-cm2 roller bottles at least 7 days prior to initiating an experiment. Portions containing cultures were routinely examined to exclude microbial contamination.

Sufficient sphingomyelin (specific activity 0.7–1.0 Ci/mol) to make a final medium concentration of 1 μmol/ml was dissolved in chloroform:methanol (2:1, v/v) and transferred under sterile conditions to the incubator. After drying under a stream of filtered N2, the lipid was covered with minimal essential medium plus penicillin and streptomycin. Vortex-mixed at 50°C for 10 min, then ultrasonicated in a bath-type ultrasonic cleaner (Cole Palmer Model 8845-30) at 45–50°C for 2 h. The medium plus suspended lipid was pooled, filtered, and made 1 mol/ml. The suspension was heated at 37°C for 1 h and filtered. The filtrate was then twice with washed (19) with one-fifth volume of 0.1 M NaCl and 0.05% Tween 80. The liposome size range from >3 to <0.22 μm by filtration (17) had very little effect on the amount of cell-associated label. Accordingly, unless specified otherwise all experiments were performed with confluent cultures with similar cell protein concentrations, incubated in 10% fetal bovine serum, and with sphingomyelin liposomes formed by sonication briefly to produce a homogenous suspension, and portions were taken for protein (18) and determination of total radioactivity. The remaining sonicated material and any medium to be analyzed was lyophilized.

0.85% NaCl solution (0.5 ml, 0.15 M) and chloroform:methanol (2:1, 5 ml) were added to the lyophilized material. The resulting suspension was heated at 37°C for 1 h and filtered. The filtrate was washed (19) with one-fifth volume of 0.1 M KCl containing 25 mg of phosphocholine, and 25 mg of choline, 0.1 M NaCl, and suspended in 0.15 M NaCl. The suspension was sonicated to produce a homogenous suspension, and portions were taken for protein (18) and determination of total radioactivity. The remaining sonicated material and any medium to be analyzed was lyophilized.

All radioactivity determinations were corrected for quenching by external standard or channels ratio methods.

The medium was treated in the same manner as the cells except that a small amount of [methyl-32P]sphingomyelin was added to the extraction medium to control for contamination of water-soluble products by the large quantities of [methyl-H]sphingomyelin in the medium. Portions of the upper phases were chromatographed on thin layers of cellulose C or silica gel G as described earlier.

Incorporation and Retention of [3H]Thymidine—Control and NP-A fibroblast cultures were grown in medium containing [3H]thymidine for 48–96 h, then in medium with cold thymidine (0.16 μmol/ml) for 48 h. Then the labeled cells were used for sphingomyelin uptake studies. [3H]Thymidine in DNA in either cells or medium was measured according to Peterson and Rubin (20).

Other Methods—Sphingomyelinase activity and tissue lipid concentrations were measured as described previously (7, 11).

RESULTS

Characteristics of Cell Types Used in This Study—The two NP-A cell lines used in this study had acid sphingomyelinase activities <4% of control when measured in vitro (Table I). Activities were not changed significantly by incubation of cells with culture sphingomyelin (1 μmol/ml of medium) for up to 120 h. There was a modest increase in cholesterol and phospholipid in the Niemann-Pick cells, and among the phospholipids, sphingomyelin was increased. Throughout these experiments, both mutant cell lines were used; there were no appreciable differences between them.

Incorporation of 32P, or [3H]Choline—There was no incorporation of either isotope into phospholipids when incubation was with medium in the absence of cells. 32P incorporation into sphingomyelin and lecithin was similar in control (Fig. 1A) and NP-A (Fig. 1B) cells. When the labeled medium was replaced with unlabeled medium at 120 h, loss of lipid label was observed in both cell types. There was a slightly greater retention of sphingomyelin label in the NP-A cells at later times (240 and 280 h), but since lecithin label was also retained (Fig. 1B), this may be a generalized phenomenon rather than the storage of a particular cell lipid. Labeled lipid in the medium was <20% of the cell-associated lipid and was not selectively enriched in labeled sphingomyelin in the NP-A medium at any time point examined (144, 240, and 288 h). Labeling of sphingomyelin was maximal during the chase period, suggesting continued labeling from some other 32P-containing fraction, such as lecithin.

Many of the same trends were observed when the cells were incubated with [3H]choline (Fig. 2). Sphingomyelin became

<table>
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<tr>
<td>Phospholipid content and sphingomyelinase activity of cultured cells from control and NP-A subjects</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>%</td>
</tr>
<tr>
<td>Phospholipida</td>
</tr>
<tr>
<td>Lysophosphatidylethanolamine</td>
</tr>
<tr>
<td>Sphingomyelnase</td>
</tr>
<tr>
<td>Phosphatidylserine + phosphatidylinositol</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
</tr>
<tr>
<td>in mg protein/h</td>
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| 118 | 159 | 5 | 4 |

a Phospholipid P content of control and NP-A cells were 6–8 and 8–12 μg/mg of protein, respectively. Lipid extracts were subjected to thin layer chromatography, and the phosphorus content of the separated lipids was determined. Recovery of phospholipid P was >95%.

b The phospholipid composition of cell line GM-112 is taken from Fishman et al. (39).
incubated for 48-120 h with or without heat-denatured cells or in medium which had been in contact with normal or NP-A cells for 4-5 days. Accordingly, the changes reported in the presence of cells are clearly cell-mediated. Cell-associated lipid was not merely adsorbed to the cell surface as there was no decrease in cell labeling when the cells were harvested by trypsin treatment rather than by scraping, a manipulation that should decrease adsorbed lipid (21).

Both control and NP-A cells take up [methyl-3H]sphingomyelin, and the levels of sphingomyelin radiolabel attained in the mutant cells were 3-6 times the levels in the control cells (Fig. 3). The levels of labeled sphingomyelin fell somewhat during the chase, but always remained higher in the NP-A cell. A substantial portion of the [3H]choline of the sphingomyelin appeared in lecithin (Fig. 3, middle; note changes in scale on the ordinate). In control cells, this amounted to 50% of the total cell-associated label by 20 h. In NP-A cells, the process was slower and only 20% of the total cell choline label was associated with lecithin by 40 h. Identity of the labeled lecithin was confirmed by chromatography in three solvent systems. Following phospholipase C hydrolysis of the lecithin, the radioactivity was water-soluble and chromatographed as a mixture of choline and phosphorylcholine.

There was also a substantial accumulation of water-soluble radioactivity in the medium (Fig. 3, right), which chromatographs as a mixture of choline and phosphorylcholine. (Salts are also extracted from the medium and interfere with the resolution between choline and phosphorylcholine in the chromatographic systems. While the label chromatographs labeled somewhat more slowly in the NP-A cell, and the radioactivity continued to increase after substitution of cold medium at 120 h (Fig. 1B). The label lost from the cell appeared in the medium, and there was no major difference in the distribution of this label between control and NP-A cells. Between 83 and 92% of the label was water-soluble and chromatographed as choline or phosphorylcholine on TLC. The remaining label chromatographed as lecithin and sphingomyelin: 60-80% as lecithin in the early postlabeling stages (between 129 and 192 h) and 10-13% in the later stages (between 192 and 288 h).

The loss of labeled lipid from control and NP-A cells was not due to cell death and dissolution into the medium. Media from cultures prelabeled with [3H]thymidine and then incubated for 48-120 h with or without exogenous sphingomyelin loading contained less than 1% of the labeled cell DNA. A similar low level of medium DNA was observed in cultured cells loaded with sphingomyelin for 120 h and maintained in culture with two medium changes for 288 h.

Although these endogenous labeling studies clearly demonstrated phospholipid metabolism in the control and mutant cell types, they were less satisfactory for studying sphingomyelin metabolism because the high rates of synthesis and turnover of other phospholipids, particularly lecithin, tended to obscure the contributions from sphingomyelin metabolism. This led us to study the uptake and metabolism of exogenous labeled sphingomyelin.

**Incorporation of Exogenous [methyl-3H]Sphingomyelin—**

The labeled exogenous sphingomyelin was not changed by incubation at 37°C for periods up to 120 h in medium with or without heat-denatured cells or in medium which had been in contact with normal or NP-A cells for 4-5 days. Accordingly, the changes reported in the presence of cells are clearly cell-mediated. Cell-associated lipid was not merely adsorbed to the cell surface as there was no decrease in cell labeling when the cells were harvested by trypsin treatment rather than by scraping, a manipulation that should decrease adsorbed lipid (21).

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with both compounds, the relative proportion of each is less certain. The capacity of both cell lines to carry out this hydrolysis is substantial, and in this particular experimental series, the NP-A cells exceeded the controls. The rapid drop in medium radioactivity following the chase at 20 h indicates that the source of the medium water-soluble label, while cell-mediated, is the [methyl-3H]sphingomyelin in the medium. If it were not, the continued hydrolysis of cell sphingomyelin and lecithin should rapidly deplete the intracellular labeled material, which does not happen (Fig. 3, left and middle). As noted earlier in the experiments with [32P] and [3H]choline labeling, a small portion of the medium radioactivity was in lipid derived from the cells. This was not examined further in these experiments because of technical uncertainties introduced by the large amounts of labeled lipid substrate already present in the medium.

**Incorporation of [choline-methyl-3H]- and [32P]sphingomyelin**—Similar results to those shown in Fig. 3 were obtained when the incubation of cells with labeled sphingomyelin and subsequent chase were extended out to 120 and 168 h, respectively. An example of a 48-h pulse and a 48-h chase is shown in Fig. 4. The top shows the results of 3H analysis (note changes in scale on the ordinate). These confirmed and extended the patterns shown in Fig. 3, although in this case the release of water-soluble label to the medium was similar for the NP-A and control cells. An additional point of interest is that with extended incubation (up to 96 h), the amount of label in lecithin in the NP-A cells continued to increase. By contrast, there was a more rapid increase in the amount of label in lecithin by 48 h in control cells, and no further increase was observed with further incubation.

An additional feature of this experiment was the use of [N-palmitoyl-1-14C]sphingomyelin, and the distribution of the 14C label is shown in the bottom of Fig. 4. The accumulation of 14C-palmitoylsphingomyelin confirms the pattern observed with the [3H]choline label as levels of label in the NP-A cells are over twice those of the controls. However, the absolute amounts of [14C]sphingomyelin retained are always greater than [3H]sphingomyelin, demonstrating that either the [14C]ceramide or the [14C]palmitic acid is being conserved and used for form sphingomyelin. That part of this may be [14C]palmitic acid is attested to by the appearance of label in lecithin (Fig. 4, middle). Again, the appearance of label is slower in the NP-A cells, suggesting that the sphingomyelin precursor is less easily degraded. The major 14C label in the medium (other than unreacted substrate at the 24- and 48-h points) is ceramide, and the amounts are close to the amounts of choline and phosphorylcholine. Free fatty acid and other labeled lipid products were less than 10% of the ceramide label.

**Incorporation of [choline-methyl-3H]- and [32P]sphingomyelin**—To obtain additional evidence that the labeled sphingomyelin was taken up intact by the cells and to determine whether the lecithin label was from the phosphorylcholine or choline moieties of sphingomyelin, we incubated control and NP-A cells with [choline-methyl-3H]- and [32P]sphingomyelin for varying time periods to 72 h. Label in the medium following incubation was in substrate, choline, phosphorylcholine, and P. The 32P/3H ratio in the cell sphingomyelin (Table II) was similar to the exogenous sphingomyelin added to the cell. This observation and the data in Fig. 4, where [N-palmitoyl-1-14C]sphingomyelin incorporation was examined, indicate that most of the labeled cell-associated sphingomyelin is taken up intact and not degraded, and then resynthesized in the cell. The 32P/3H ratio in lecithin is similar to the sphingomyelin, and any changes are relatively modest, suggesting that most of the labeled lecithin is formed from the intact phosphorylcholine moiety of sphingomyelin.

Another possible explanation for the similar 32P/3H ratio of the phosphorylcholine of sphingomyelin and lecithin is that the phosphorylcholine hydrolyzed from sphingomyelin was further hydrolyzed to P and choline and reused for lecithin synthesis. In such circumstances, the pool sizes of P and choline would have to be similar. This is not the case in the medium where there is a 100-fold excess of PO4 as compared to choline, and seems improbable in the cell. An alternative possibility is that the phosphorylcholine that comprises a portion of the water-soluble label released to the medium is the immediate precursor of cellular lecithin. This possibility was examined.

**Incorporation of [methyl-3H]- and [32P]phosphorylcholine**—Control and NP-A cell were incubated with [methyl-3H]- and [32P]phosphorylcholine (1.7 μmol/ml) for 24, 48, and 120 h. After harvest, the amount of cell-associated radioactivity was determined. The incorporation of 3H from phosphorylcholine was less than 10% of that observed when [methyl-3H]choline was used to label the cells. Furthermore, the incorporation of 32P and 3H in sphingomyelin and lecithin of control and NP-A cells grown in medium containing [choline-methyl-3H]- and [32P]sphingomyelin

<table>
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<tr>
<th>Cells</th>
<th>Time (h)</th>
<th>32P</th>
<th>3H</th>
<th>32P/3H</th>
<th>32P</th>
<th>3H</th>
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<tr>
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<td>24</td>
<td>4,050</td>
<td>17,100</td>
<td>0.24</td>
<td>3,050</td>
<td>11,500</td>
<td>0.25</td>
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<td>48</td>
<td>5,590</td>
<td>24,900</td>
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<td></td>
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<td>6,460</td>
<td>30,800</td>
<td>0.21</td>
<td>6,174</td>
<td>37,950</td>
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<tr>
<td>NP A</td>
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<td>55,100</td>
<td>0.13</td>
<td>1,440</td>
<td>7,180</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>13,800</td>
<td>108,000</td>
<td>0.13</td>
<td>2,160</td>
<td>19,300</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>14,310</td>
<td>121,000</td>
<td>0.12</td>
<td>2,780</td>
<td>55,300</td>
<td>0.08</td>
</tr>
</tbody>
</table>

*Total disintegrations/min in lecithin or sphingomyelin extracted from the cells with chloroform:methanol and separated by TLC.
Mean cell lipid among NP-cells (Fig. 5). Since incorporation into the medium (final concentration of diglyceride to lecithin is a major fate of 3H label in the control cells, moving from sphingomyelin to lecithin in control cells, and could be stimulated 9-fold by the addition of more choline to the external portion of the lipid bilayer, it is tempting to speculate that this activity is located at or near the cell surface and that it may play an important role in intercellular interactions, such as cell contact and recognition.

**Cellular Hydrolysis and Transfer of the Sphingomyelin Phosphorylcholine to Lecithin**—Maziere et al. (35) have recently reported that [choline-methyl-14C]sphingomyelin was taken up and degraded by cultured fibroblasts and that 65–80% of the cell-associated lipid label was in lecithin. Although NP-A cells were examined also, they did not observe either storage or degradation of sphingomyelin or the appearance of label in lecithin. The reasons for this difference between our studies may relate to differences in substrate concentration, specific activity, and method of suspension (1 μmol/ml, 1 μCi/μmol as an ultrasonically prepared liposomal suspension in our case versus 0.002 μmol/ml, 44 μCi/μmol delivered in ethanol). The label in lecithin was not further examined by us and to our knowledge, the present study is the first direct demonstration that sphingomyelin is a precursor of the phosphorylcholine moiety of lecithin (Figs. 3 and 4 and Table II).

The mechanism of transfer of the phosphorylcholine group is not known, but there are at least three possibilities. First, sphingomyelin may be hydrolyzed by the lysosomal sphingomyelinase to phosphorylcholine and ceramide. The phosphorylcholine enters the intracellular pool of phosphorylcholine in the Hurler and Hunter syndromes (27, 28), cerebroside sulfate in metachromatic leukodystrophy (29), and glucosylceramide in Gaucher’s disease (30). While there is certainly evidence for abnormal kinetics of sphingomyelin turnover and storage (Figs. 3 and 4) in the mutant cells, there is also substantial metabolism. The nature and location of this metabolism suggests at least two other pathways for sphingomyelin degradation in the cultured human fibroblast in addition to the lysosomal sphingomyelinase.

**Lyosomal Hydrolytic Activity**—This activity is clearly at very low levels in the NP-A cells (Table I), and its deficiency probably contributes to the abnormal cellular accumulation of labeled sphingomyelin in the mutant cells (Figs. 3 and 4 and Ref. 31). The abnormal cellular accumulation only appears when the cells are loaded with exogenous substrate; under normal conditions, endogenous sphingomyelin catabolism is relatively normal (Figs. 1 and 2). This might be attributed to exchange or secretion of phospholipid by cultured cells to the medium as observed by us and others (20). On the other hand, observations that the sphingomyelin accumulation in Niemann-Pick disease is predominantly in cells with extensive endocytotic activity, such as macrophages, Kupffer cells, etc. (2), strongly suggest that the lysosomal pathway plays a major role in these circumstances only. For cells less active in endocytosis, other pathways predominate.

**Other Hydrolytic Activities**—In the presence of sphingomyelin liposomes in the medium, both NP-A and control cells degrade sphingomyelin to phosphorylcholine and choline and ceramide. The activity is cell- and liposome-dependent and clearly separate from the internal sphingomyelin and lecithin. If the latter were the substrates, the rates of hydrolysis would quickly deplete the intracellular labeled material, which does not happen. Whether the liposomal lipid exchanges with membrane lipid or is part of a pinocytic compartment separate from the stored internal lipid is not known. The activity cannot be demonstrated in vitro under conditions optimal for the neutral Mg2+-stimulated brain sphingomyelinase (7); whether it is another sphingomyelinase (32, 33), a cell-surface phospholipase C (34), or some other hydrolytic activity remains to be determined. Since choline-containing lipids are the major plasma membrane lipids, particularly in the external portion of the lipid bilayer, it is tempting to speculate that this activity is located at or near the cell surface and that it may play an important role in intercellular interactions, such as cell contact and recognition.

**DISCUSSION**

The two NP-A cell lines (318 and GM-112) used in this study show a profound deficiency of sphingomyelinase (<96%) and a modest increase in sphingomyelin. These findings clearly indicate that the mutant phenotype is expressed in culture. Accordingly, we expected to see abnormal kinetics of intracellular accumulation and/or loss of sphingomyelin in cultured fibroblasts as demonstrated for mucopolysaccharides in the Hurler and Hunter syndromes (27, 28), cerebroside sulfate in metachromatic leukodystrophy (29), and glucosylceramide in Gaucher’s disease (30). While there is certainly evidence for abnormal kinetics of sphingomyelin turnover and storage (Figs. 3 and 4) in the mutant cells, there is also substantial metabolism. The nature and location of this metabolism suggests at least two other pathways for sphingomyelin degradation in the cultured human fibroblast in addition to the lysosomal sphingomyelinase.
and is available for the CTP-dependent pathway of lecithin biosynthesis. The slower appearance of phosphorylcholine in the NP-A cell reflects the very low levels of lysosomal sphingomyelinase. The reduction in lecithin labeling when extra choline is added to the medium (Fig. 5) may reflect an increase in the intracellular phosphorylcholine pool and a consequent reduction in its specific activity.

The second possibility is a direct enzyme-mediated transfer of phosphorylcholine from sphingomyelin to diglyceride. The opposite reaction, the transfer of phosphorylcholine from lecithin to ceramide, has been documented with mouse liver microsomes (23, 25), SV40-transformed and normal mouse fibroblasts, and plasma membrane and Golgi fractions of SV40-transformed mouse fibroblasts (22, 24, 26). In all cases, the transfer is catalyzed by and may take place within the membrane fractions. Fusion and transfer protein-dependent and -independent incorporation of intact lipids into membranes is well documented (21), probably takes place during our experiments, and would make radioactive substrate available to the phosphorylcholine transfer process. The delay in and lower levels of phosphorylcholine transfer in the NP-A cells (Figs. 3 and 4) could be due to impairments in sphingomyelinase since its activity is not affected by endocytosis, but also by direct incorporation into cellular membranes. The evidence for endocytosis is the storage in the lysosome, the substantial activity in the sphingomyelinase-deficient NP-A cells suggests a more direct relationship.

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Pathways of sphingomyelin metabolism in cultured fibroblasts from normal and sphingomyelin lipidosis subjects.
M W Spence, J T Clarke and H W Cook


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