Niemann-Pick type C disease (NPC) belongs to the group of lysosomal storage diseases characterized by an accumulation of cholesterol and sphingomyelin. Using a mutant mouse strain, enzymatic markers for lysosomes, mitochondria, microsomes, and peroxisomes were investigated in the liver and brain. Aside from lysosomal changes, we found a sizable decrease of peroxisomal \( \beta \)-oxidation of fatty acids and catalase activity in the brain and liver. Isolated peroxisomes displayed a significant decrease of these enzyme activities. Furthermore, the only phospholipid change in brain was a decreased content of the plasmalogens of C₁₄, C₁₆, and C₁₈, and the dimethylacetel pattern was also modified. The electron microscopical appearance of peroxisomes did not display any large changes. The defect of peroxisomal enzymes was already present 18 days before the onset of the disease. In contrast, the lysosomal marker enzyme increased in activity only 6 days after appearance of the symptoms. The events of the studied process have previously been considered to be elicited by a lysosomal deficiency, but this study demonstrates disturbances similar to those in a number of peroxosomal diseases. It appears that the peroxisomal impairment is an early event in the process and could be a factor in the development of Niemann-Pick type C disease.

Not only are the contents of the mevalonate pathway lipids and sphingomyelin modified in the disease, several other lipids are also changed, particularly in the liver. Bis(monoacylglycerol)phosphate, glycosylceramide, and sphingoid bases are reported to be elevated (5–7). In the brain, abnormalities are more restricted and mainly affect glycolipids. A severalfold increase of glycosylceramide and lactosylceramide and an increase of GM₂ and GM₃ gangliosides have been found. In the liver of mutant mice the amount of sterol carrier protein 2, a protein suggested to be involved in cholesterol transport and biosynthesis, is decreased by 80% (8). Most of the various lipid accumulations have been associated with lysosomes, and the levels of several lysosomal enzymes were found to be altered (9).

The extensive modifications in cholesterol and dolichol metabolism and transport raise several questions concerning the biochemical etiology of this disease. These two lipids are synthesized not only in microsomes but also in peroxisomes and are subjected to independent regulations (10–12). In this study, we have therefore investigated the possibility that peroxisomes are also modified in Niemann-Pick type C disease, which is generally considered to be elicited by an enhanced lysosomal storage of lipids. The analyses led to the novel finding that in this condition, in contrast to the lysosomal disorders, a deficiency of peroxisomal function is an early event.

### MATERIALS AND METHODS

**Animals**—In all experiments, a mutant BALB/c mouse model of NPC was used. The parental strain, from which the mutant mice were derived, serves as a control. The controls were age-matched to the diseased mice. Since NPC is an autosomal recessively inherited disease, only 25% of the mice develop pathological symptoms. The most prominent symptom is ataxia, which appears around 45–50 days of age. The mice showing ataxia were selected for investigation within 3 days.

**Preparation of Homogenate and Subfractionation**—After decapitation, tissues were quickly removed and homogenized in 0.25 M cold sucrose (1 g/ml) with a Potter–Elvehjem homogenizer. The homogenates were centrifuged at 105,000 × g for 15 min to obtain the heavy mitochondrial/lysosomal fraction (13). The supernatant was centrifuged at 25,300 × g for 15 min, and the pellet was the light mitochondrial/lysosomal fraction. The supernatant above the latter pellet was centrifuged at 150,000 × g for 60 min to obtain the microsomal fraction.

**Enzyme Assays**—Peroxosomal fatty acyl-CoA oxidase activity was determined using lauroyl-CoA, palmitoyl-CoA, or arachidonyl-CoA as substrates. The H₂O₂ produced was quantified fluorometrically by following the horseradish peroxidase-catalyzed oxidation of 4-[4-diethylamino]phenyl acetic acid into 6,6'-dihydroxy-(1,1'-biphenyl)-3,3'-diacetic acid (15).

**References and Notes**

(1) This work was supported by grants from the Swedish Medical Research Council and the Swedish Cancer Society. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

(2) To whom correspondence should be addressed. Tel.: 46-8-162445; Fax: 46-8-153679; E-mail: fiia@biokemi.su.se.

(3) The abbreviations used are: GM₂, ceramide-glucose-galactose-[(1,4)-diacetic acid; NPC, Niemann-Pick type C; FA, fatty acid; PE, phosphatidylethanolamine; DMA, dimethyl acetal; FAME, fatty acyl methyl ester; HPLC, high performance liquid chromatography; PPAR, peroxisome proliferator activated receptor.
Peroxisomes and Niemann-Pick Type C Disease

Enzyme activities in liver homogenate

The homogenates were prepared 2 days after appearance of the symptoms. NADPH-cytochrome c reductase was determined as a microsomal marker; cytochrome oxidase was employed as a marker for the mitochondria; and urate oxidase, catalase, and lauroyl-CoA oxidase were the peroxisomal markers. The values are the means ± S.D. of four separate experiments, using individual mice. * p < 0.05 *** p < 0.001.

**Table I**

| Enzyme                        | Control | Liver  |
|-------------------------------|---------|--------|
| NADPH-cytochrome c reductase  | 3.31 ± 0.41 | 3.37 ± 0.33 |
| Cytochrome oxidase             | 0.133 ± 0.015 | 0.154 ± 0.016 |
| Urate oxidase                 | 0.070 ± 0.005 | 0.057 ± 0.006* |
| Catalase                      | 0.406 ± 0.053 | 0.297 ± 0.028* |
| Lauroyl-CoA oxidase           | 0.891 ± 0.120 | 0.437 ± 0.028*** |

* nmol cytochrome c reduced/(min × mg protein).
* μmol cytochrome c oxidized/(mg × min).
* μmol urate/(mg × min).
* nmol H2O2/(mg × min).
* nmol/(min × mg protein).

Enzyme Composition—Several enzyme activities, known to be associated with specific organelles, were analyzed in the liver homogenates of mice exhibiting clinical signs of NPC disease, at the initial phase, around 48–50 days of life. The specific activity of NADPH-cytochrome c reductase, an enzyme present only in the endoplasmic reticulum, was identical in diseased mice and the age-matched controls (Table I). The activity of cytochrome oxidase, a component of the inner mitochondrial membrane, was also unaltered. On the other hand, three enzymes associated with peroxisomes exhibited reduced activity in comparison with the controls. There was a moderate decrease of urate oxidase and catalase activities (20–30%), and the β-oxidation enzyme, lauroyl-CoA oxidase, activity was decreased by 50%.

Differential centrifugation was performed to obtain a heavy mitochondrial/lysosomal fraction and a light mitochondrial/lysosomal fraction, as well as microsomes. The mitochondrial fractions contained the peroxisomes. The peroxisomes present in the two fractions exhibited different properties (Table II). The specific activities of both catalase and lauroyl-CoA oxidase were greatly decreased in the heavy mitochondrial fraction of the diseased mice, while the decrease was quite limited in the light mitochondrial fraction. The specific activity of acid phosphatase was doubled in the two mitochondrial fractions from diseased mice, which is in agreement with the finding that lysosomes are affected in NPC disease. There was also a 3-fold increase of acid phosphatase activity in the microsomes, which may be explained by the slow sedimentation of a fraction of lipid-filled lysosomal vesicles remaining in the microsomal fraction. Both cytochrome oxidase and NADPH-cytochrome c reductase activity remained constant when compared to control mice, indicating that neither the mitochondria nor the endoplasmic reticulum are affected by the disease.

In order to verify the defects at the organelle level, peroxisomes were isolated using Nycodenz gradients (Table III). Using this procedure, the fraction obtained has a very low cross-contamination with other organelles but the recovery is low and prevents extensive investigation. The isolated peroxisomes exhibited 40–50% decreased urate oxidase, catalase, and lauroyl-CoA oxidase activities when the isolated fractions from NPC mice were compared to the control.

Brain homogenates were also analyzed for peroxisomal activities. Catalase activity, also in this tissue, was found to be decreased (Table IV). Since brain peroxisomes preferentially oxidize long chain fatty acids, arachidonoyl-CoA oxidase was measured and was found to be decreased by as much as 70% in diseased animals.

**Peroxisomal Oxidation of Various FA**—The peroxisomal β-oxidation of FA in the liver homogenates was determined by measuring H2O2 production in the presence of various FA-CoA species (Fig. 1). The acyl-CoA oxidase activity measured with octanoyl-, lauroyl-, myristoyl-, palmitoyl-, and arachidonoyl-CoA as substrates was decreased by half or more as compared to the control, indicating a generalized impairment of peroxisomal β-oxidation.

**Developmental Changes**—Symptoms of the disease become apparent around 48 days of age, and the mice die at around 70 days of age.
The average age of the mice was 31 days, 18 days before the appearance of the disease symptoms. When the symptoms first appeared, palmityl-CoA oxidation and lauroyl-CoA oxidase as well as catalase and urate oxidase activities were greatly diminished, but activities gradually recovered and almost reached the level of control values by the end of the 3rd week (Fig. 2). In contrast, lysosomal acid phosphatase activity was about the same as the control during the first few days and reached maximal amplification 13 days later.

The experiment above suggested that the peroxisomal enzyme activities may already be decreased before the onset of overt clinical symptoms. Groups of mice pooled into affected and nonaffected were selected for analysis of the liver (Table V). The average age of the mice was 31 days, 18 days before the symptoms are expected to appear. Mice exhibiting elevated levels of cholesterol and dolichyl phosphate were considered as homozygous for the NPC mutation. This group of animals exhibited a considerably decreased activity of peroxisomal β-oxidation, indicating that the peroxisomal deficiency is manifested long before the appearance of the disease symptoms.

**Electron Microscopy of the Liver Peroxisomes—Hepatocytes** contain morphologically well characterized peroxisomes with a rich proteinaceous content in the lumen. We have used a diaminobenzidine staining procedure, which detects peroxisomal catalase as a granular precipitation (data not shown). No obvious differences in appearance and distribution could have been observed.
be observed between control and NPC liver. Investigating a series of images from both control and diseased tissues, we could not see any great differences in the number or size of these organelles.

**Phospholipids of the Brain and Liver—**

The major phospholipid components of the mouse brain, PE and phosphatidylcholine, are present in about equal amounts (Fig. 3A). The brain is also relatively rich in phosphatidylserine and sphingomyelin, while cardiolipin and phosphatidylinositol are smaller components. No clear-cut difference in total amount of phospholipid could be observed between control and NPC mouse brain. On the other hand, after phospholipid class separation there was a small decrease of PE content. PE consists of two main sub-

classes, the alkylacyl and the diacyl forms (Fig. 4). The alkylacyl PE, the precursor of the alkenylacyl form, makes up only a few percent of the total. In the diseased brain, the amount of alkenylacyl PE was decreased by one third, while the levels of the two other forms were unchanged. Thus, the decrease of PE in NPC brain is solely caused by a decrease of PE plasmalogen. Some increase of sphingomyelin was also noticed, but this change was not statistically significant.

In NPC liver, the total phospholipid level on DNA basis was increased from 12.89 ± 1.23 to 15.71 ± 1.05 mg of phospholipid/mg of DNA. This is explained by the severalfold elevation of the sphingomyelin content (Fig. 3B). The dominating phospholipid, phosphatidylcholine, as well as cardiolipin, phosphatidylinositol, and phosphatidylserine, all remained unchanged.

**Fatty Acid Composition—**

The fatty acid patterns of total lipids in the brain and liver are very different (Table VI). Dimethyl acetals (DMAs) are the aldehyde associated with carbon 1 on the plasmalogens. They are present in the liver in only minute amounts because of the very low level of plasmalogens in this tissue and therefore were not determined. In both organs, the FA composition of total lipids remained completely unchanged in the diseased state. In the brain, DMAs are restricted to a few components, consisting of 16:0, 18:0, and 18:1. There were significant changes in DMA distribution in the case of NPC brain, apparent as an increase of the 16:0 and a decrease of the 18:1 species.

Because of the observed modifications of the DMA pattern, we also analyzed the FA composition of PE subclasses (Table VII). The plasmalogen form was highly enriched in polyunsaturated FA, while the diacyl form was dominated by the 16:0 and 18:0 species. The FA composition of alkenylacyl and diacyl PE was very similar in both control and NPC mice. The DMA composition of the plasmalogen fraction of the PE was, as expected, similar to that found for the total lipids. In the diseased state, 16:0 was elevated, 18:1 decreased, and 18:0 remained unchanged.
The C-type Niemann-Pick disease represents a deviation from other forms of NP, since it is dominated by cholesterol accumulation in the lysosomes, caused by a lesion in the intracellular trafficking of endocytosed cholesterol. The murine model of the disease makes possible a detailed study of the enzyme modifications involved. Using this system, we have found impairments in peroxisomal activities, indicating the involvement of this organelle in the pathogenesis of this disorder. These observations reveal new aspects of the disease process, as they demonstrate that peroxisomal deficiencies occur long before the manifestation of the disease symptoms.

There was a sizable decrease of all peroxisomal enzyme activities determined in the liver, i.e. β-oxidation of various types of fatty acids, catalase and urate oxidase. The impairment of β-oxidation and catalase was also expressed in the brain of these mice, indicating involvement of peroxisomal dysfunction in multiple organs. Furthermore, the only change in brain phospholipids was a 33% decrease of PE plasmalogens. The initial portion of plasmalogen synthesis, formation of the ether bond, takes place in peroxisomes (28), which explains why only plasmalogen and not other phospholipids are affected. The dimethyl acetal pattern of PE plasmalogens was also substantially modified, and, consequently, the decrease in PE plasmalogens is probably caused by an impaired peroxisomal synthesis rather than increased breakdown.

Some peroxisomal diseases, such as Zellweger syndrome, are characterized by accumulation of very long chain fatty acids (29). In these cases, the peroxisomal β-oxidation is completely missing. In NPC mice, the β-oxidation is only partially deficient and no changes in the lipid-bound fatty acids could be observed. Thus, it appears that the residual β-oxidation capacity is sufficient enough, which, together with the relatively short life span of the diseased mice, prevents accumulation of very long chain fatty acids.

Electron microscopical investigations did not reveal great changes in the structure and the number of peroxisomes in the diseased mice. This finding is not surprising since in many identified peroxisomal diseases where partial defects of enzyme systems have been observed, the peroxisomal morphology remained unchanged (30). This fact, however, does not exclude the possibility that structural modifications at the organelle level exist. In our experiments, the peroxisomes present in the heavy mitochondrial fraction displayed considerable enzyme deficiencies in contrast to those present in the light mitochondrial fraction. This suggest that a unique population of this organelle may be responsible for the anomalies found in the disease process.

Isolated peroxisomes exhibit a 40–50% decrease of various peroxisomal enzyme activities, and it would be of considerable interest to perform subfractionation studies to investigate the possible heterogeneity in this fraction. Such a subfractionation study, however, has not yet been performed mainly because of the small number of peroxisomes in the liver and the extremely poor recovery afforded by the present methods employed.

It has previously been found that in the NPC mouse liver, sterol carrier protein-2 exhibits an 80% decrease in the post-nuclear fraction (8). This protein is believed to be necessary in certain reactions, including the transformation of lanosterol to cholesterol (31), the activation of the microsomal cis-prenyltransferase (32), and the transport of steroids (33). In the liver, the major part of this protein is present in peroxisomes and the rest is a cytosolic component (34). The large decrease of the level of sterol carrier protein-2 in NPC lends further support to the participation of peroxisomes in the cellular pathogenic process.

**Fig. 3. Phospholipid composition of the brain and liver.** NPC organs were taken from mice 4–6 days after the development of symptoms. Lipids were extracted from brain and liver homogenates, and the phospholipid classes were separated by HPLC on a silica column. Quantification was performed by determining lipid phosphorus. A, phospholipids from brain; B, phospholipids from liver. The values are the means of five separate experiments. The bars show S.D.; **, p < 0.01.

**Fig. 4. Subclasses of PE in mouse brain.** NPC mice were taken 4–6 days after the appearance of symptoms for preparation of brain homogenates. After isolation of PE, the lipid was hydrolyzed by phospholipase C and acetylated in the presence of pyridine. The alkenylacyl-, alkylacyl-, and diacyl forms were separated on a silica column by HPLC. Quantification was achieved by gas chromatography of the fatty acid methyl esters. The values are the means of separate experiments, using 4 individual mice from each group. The bars show S.D.; **, p < 0.01.
Peroxisomes and Niemann-Pick Type C Disease

The total lipid extracts of the brain and liver were treated with boron trifluoride in methanol in order to prepare fatty acyl methyl esters and dimethyl acetals. Separation and quantification of the individual components was achieved by gas chromatography. The values are the means of five experiments, using separate mice. S.D. values of the means were 3–6%. **, p < 0.01.

|       | NPC  | Control | NPC  | Control |
|-------|------|---------|------|---------|
| FAME  |      |         |      |         |
| 16:0  | 27.17| 24.73 | 22.07| 22.09 |
| 16:1  | 0.74 | 1.09  | 1.11 | 0.84   |
| 18:0  | 20.02| 19.50 | 14.32| 13.90 |
| 18:1  | 17.54| 19.91 | 13.73| 14.19 |
| 18:2  | 1.17 | 0.78  | 19.06| 19.72 |
| 18:3  | 0.24 | 0.24  | 0.46 | 0.47   |
| 20:1  | 1.19 | 0.75  | 0.54 | 0.39   |
| 20:4  | 10.13| 9.25  | 17.20| 15.85 |
| 22:4  | 2.63 | 3.14  | 1.09 | 0.58   |
| 22:6  | 19.34| 17.15 | 9.56 | 10.14 |
| 24:0  | 0.59 | 0.93  | 1.01 | 0.52   |
| 24:1  | 0.78 | 1.20  | 1.16 | 1.24   |
| DMA   |      |        |      |         |
| 16:0  | 33.01**| 28.83  |      |         |
| 18:0  | 42.65 | 42.66 |      |         |
| 18:1  | 24.33**| 28.53  |      |         |

|       | NPC  | Control | NPC  | Control |
|-------|------|---------|------|---------|
| FAME  |      |         |      |         |
| 16:0  | 4.80 | 4.44   | 12.68| 13.10  |
| 18:0  | 2.19 | 2.09   | 8.99 | 7.66   |
| 18:1  | 20.32| 20.04  | 38.17| 38.38  |
| 20:1  | 5.62 | 5.49   | 4.51 | 4.28   |
| 20:4  | 15.05| 14.33  | 6.06 | 6.41   |
| 22:4  | 10.41| 12.51  | 8.44 | 7.07   |
| 22:6  | 41.63| 39.80  | 21.18| 21.12  |
| DMA   |      |        |      |         |
| 16:0  | 34.24**| 27.17  |      |         |
| 18:0  | 41.31 | 42.78 |      |         |
| 18:1  | 24.45**| 30.05  |      |         |

FAME and DMA compositions of PE subclasses in mouse brain

After isolation of PE the diacyl, alkenylacyl, and alkylacyl subclasses were separated by HPLC after their conversion to diradylglycerol derivatives. Fatty acyl methyl esters and dimethyl acetals were isolated by gas chromatography. Values are the means of five individual experiments; S.D. values of the means were 2–6%. **, p < 0.01.

|       | NPC  | Control | NPC  | Control |
|-------|------|---------|------|---------|
| FAME  |      |         |      |         |
| 16:0  | 4.80 | 4.44   | 12.68| 13.10  |
| 18:0  | 2.19 | 2.09   | 8.99 | 7.66   |
| 18:1  | 20.32| 20.04  | 38.17| 38.38  |
| 20:1  | 5.62 | 5.49   | 4.51 | 4.28   |
| 20:4  | 15.05| 14.33  | 6.06 | 6.41   |
| 22:4  | 10.41| 12.51  | 8.44 | 7.07   |
| 22:6  | 41.63| 39.80  | 21.18| 21.12  |
| DMA   |      |        |      |         |
| 16:0  | 34.24**| 27.17  |      |         |
| 18:0  | 41.31 | 42.78 |      |         |
| 18:1  | 24.45**| 30.05  |      |         |

Table VI

### REFERENCES

1. Vanier, M. T., Pentchev, P. G., Rodriguez-Lafrasse, C., and Rousson, R. (1991). *J. Inher. Metab. Dis.* **14**, 580–595.
2. Carstea, E. D., Polymeropoulos, M. H., Parker, C. C., Detera-Wadleigh, S. D., O’Neill, R. R., Patterson, M. C., Goldin, E., Xiao, H., Strauss, R. E., Vanier, M. T., Brady, R. O., and Pentchev, P. G. (1993). *Proc. Natl. Acad. Sci. U. S. A.* **90**, 2002–2004.
3. Pentchev, G. G., Brady, R. O., Blanche-Mackie, E. J., Vanier, M. T., Carstea, E. D., Parker, C. C., Goldin, E., and Roff, C. F. (1994). *Biochim. Biophys. Acta* **1225**, 235–243.
4. Scieder, S., Pentchev, P. G., Brunk, U., and Dallner, G. (1995). *J. Neurochem.* **65**, 670–676.
5. Pentchev, P. G., Vanier, M. T., Suzuki, K., and Patterson, M. C. (1995) in *Metabolic and Molecular Basis of Inherited Disease* (Scrivner, C. R., Beaudet, A. L., Sly, W. S., and Valle, D. D., eds) 7th Ed., pp. 2625–2639, McGraw Hill, New York.
6. Goldin, E., Roff, C. F., Miller, S., Rodriguez-Lafrasse, C., Vanier, M. T., Brady, R. O., and Pentchev, P. G. (1992). *Biochim. Biophys. Acta* **1127**, 303–311.
7. Rodriguez-Lafrasse, C., Rousson, R., Pentchev, P. G., Louiset, P., and Vanier, M. T. (1994). *Biochim. Biophys. Acta* **1226**, 138–144.
8. Roff, C. F., Putusyzen, A., Strauss, J. F., III, Billheimer, J. T., Vanier, M. T., Brady, R. O., Scallen, T. J., and Pentchev, P. G. (1992) *J. Biol. Chem.* **267**, 15902–15908.
9. Pentchev, P. G., Gal, A. E., Booth, A. D., Omodeo-Sale, F., Fousk, J., Neumeyer, B. A., Quirk, J. M., Dawson, G., and Brady, R. O. (1980). *Biochim. Biophys. Acta* **619**, 669–679.
10. Gru¨nler, J., Ericsson, J., and Dallner, G. (1994) *Biochim. Biophys. Acta* **1212**, 259–277.
11. Ericsson, J., Appelkvist, E.-L., Thelin, A., Chojnacki, T., and Dallner, G. (1992)
12. Ericsson, J., Runquist, M., Thelin, A., Andersson, M., Chojnacki, T., and Dallner, G. (1993) J. Biol. Chem. 268, 832–838
13. Runquist, M., Ericsson, J., Thelin, A., Chojnacki, T., and Dallner, G. (1994) J. Biol. Chem. 269, 832–838
14. Appelkvist, E. L., Reinhart, M., Fischer, R., Billheimer, J., and Dallner, G. (1990) Arch. Biochem. Biophys. 282, 318–325
15. Poesch, M. S., and Yamaizaki, R. K. (1986) Biochim. Biophys. Acts 884, 585–593
16. Lazarow, P. B., and DeDuve, C. (1976) Proc. Natl. Acad. Sci. U.S.A. 78, 2043–2046
17. Bergmeyer, H. U. (1955) Biochim. Z. 327, 255–258
18. Leighton, F., Poole, B., Beaufay, H., Baudhuin, P., Coffey, J. W., Fowler, S., and DuDeve, C. (1968) J. Cell Biol. 37, 482–513
19. Sottacosa, G. L., Kuylenstierna, G., Ernster, L., and Bergrenstand, A. (1967) J. Cell Biol. 32, 415–438
20. Ganoza, M. C. (1964) The Role of Lipid in Glucose-6-phosphatase. Ph.D. thesis, Duke University, University Microfilms, Inc., Ann Arbor, MI
21. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 295–375
22. Burton, K. (1956) Biochem. J. 62, 315–323
23. Dugan, L. L., Demediu, P., Pendley, C. E., II, and Horrocks, L. A. (1986) J. Chromatogr. 378, 317–327
24. Valtersson, C., and Dallner, G. (1982) J. Lipid Res. 23, 868–876
25. Nakagawa, Y., and Horrocks, L. A. (1983) J. Lipid Res. 24, 1268–1275
26. Morrison, W. R., and Smith, L. M. (1964) J. Lipid Res. 5, 600–608
27. Angermueller, S., and Fahimi, H. D. (1981) Histochemistry 71, 33–44
28. van den Bosch, H., Schutz, R. H., Wadars, R. J. A., and Tager, J. M. (1992) Annu. Rev. Biochem. 61, 157–197
29. Lazarow, P. B., and Moser, H. W. (1989) in Metabolic Basis of Inherited Disease (Scriver, C. R., Beaudet, A. L., Sly, W. S., and Valle, D., eds) 6th Ed., pp. 1479–1509, McGraw Hill, New York
30. Goldfischer, S. L. (1988) in The Liver: Biology and Pathobiology (Arias, I. M., Jakoby, W. B., Popper, H., Schachter, D., and Shafritz, D. A., eds) 2nd Ed., pp. 255–267, Raven Press, New York
31. Scallen, T. J., Shuster, M. W., and Dhar, A. K. (1971) J. Biol. Chem. 246, 224–230
32. Ericsson, J., Scallen, T. J., Chojnacki, T., and Dallner, G. (1991) J. Biol. Chem. 266, 10662–10667
33. Scallen, T. J., Pastuszyn, A., Noland, B. J., Roland, C., Kharroubi, A., and Vahouny, G. V. (1985) Chem. Phys. Lipids 38, 239–261
34. Osendorp, B. C., and Wirtz, K. W. A. (1993) Biochimie 75, 191–200
35. Dreyer, C., Krey, G., Keller, H., Grivel, F., Helftenbein, G., and Wahl, W. (1992) Cell 68, 879–887
36. Issemann, I., and Green, S. (1990) Nature 347, 645–649
37. Keller, H., Dreyer, C., Medin, J., Mahfoudi, A., Ozato, K., and Wahl, W. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 2160–2164
38. Lemberger, T., Staels, B., Saladin, R., Desvergne, B., Auwerx, J., and Wahl, W. (1994) J. Biol. Chem. 269, 24527–24530