Biochimica et Biophysica Acta, 713 (1982) 61–67
Elsevier Biomedical Press

BBA 51213

TISSUE DISTRIBUTION AND SUBCELLULAR LOCALIZATION OF PHOSPHATIDYLCHOLINE TRANSFER PROTEIN IN RATS AS DETERMINED BY RADIOIMMUNOASSAY

TOM TEERLINK [a], THEO P. VAN DER KRIFT [a], MARTIN POST [b] and KAREL W.A. WIRTZ [a]

[a] *Laboratory of Biochemistry, State University of Utrecht, Transitorium 3, Padualaan 8, NL-3584 CH Utrecht and* [b] *Laboratory of Veterinary Biochemistry, State University of Utrecht, Biltstraat 172, NL-3572 BP Utrecht (The Netherlands)*

(Received May 27th, 1982)

Key words: Phosphatidylcholine transfer protein; Radioimmunoassay; (Rat)

A radioimmunoassay for the phosphatidylcholine-transfer protein from rat liver was used to measure levels of PC-transfer protein in rat tissues. The assay as described before (Teerlink, T., Poorthuis, B.J.H.M., Van der Krift, T.P. and Wirtz, K.W.A., Biochim. Biophys. Acta 665 (1981) 74–80) was modified in order to measure PC-transfer protein in tissue homogenates and subcellular membrane fractions. To this end both a detergent (Triton X-100) and a proteolytic enzyme inhibitor (aprotinin) were added to the assay medium. The radioimmunoassay measured levels of PC-transfer protein in the range of 5–50 ng and was specific for PC-transfer protein from rat tissues. Subcellular distribution studies showed that in 10% (w/v) homogenates of liver approximately 60% of the PC-transfer protein was present in the 105000 × *g* supernatant fraction, the remainder being evenly distributed over the particulate fractions. PC-transfer protein associated with the particulate fractions was almost completely removed by a single washing step, suggesting a dynamic equilibrium between membrane-bound and soluble PC-transfer protein. Both 105000 × *g* supernatants and homogenates of various rat tissues were assayed. The highest levels of PC-transfer protein were measured in liver and intestinal mucosa. Lower values were found in kidney, spleen and lung, whereas heart and brain contained hardly any PC-transfer protein. PC-transfer protein levels in regenerating rat liver did not differ significantly from levels in normal liver. In fetal lung a change in PC-transfer protein content during development was observed, with a clear maximum 2 days before term, suggesting an involvement of PC-transfer protein in the secretion of lung surfactant.

Introduction

Eukaryotic cells contain proteins that facilitate the transfer of phospholipids between membranes in vitro [1,2]. Phospholipid transfer proteins have been fully purified from bovine liver [3,4], heart [5] and brain [6], rat liver [7–9] and hepatoma [10,11] and maize seedlings [12]. According to which phospholipid is preferentially transferred these proteins are classified as phosphatidylcholine-transfer protein [3,8], phosphatidylinositol-transfer protein [5,6], sphingomyelin-transfer protein [11] and non-specific phospholipid-transfer protein [4,7,9]. In studies on the physiological role of these proteins a number of observations have been made which suggest a relationship between the activity of these proteins and certain aspects of membrane development and phospholipid composition. An increase of phosphatidylinositol-transfer activity was observed in rat brain which coincided with the onset of myelination [13,14]. Phosphatidylcholine-transfer activities have been found to correlate with the demand for lung surfactant di-palmitoylphosphatidylcholine [15,16]. The occur-

Abbreviation: PC, phosphatidylcholine.

rence of sphingomyelin in mitochondria from rat tumors and fetal liver is thought to be due to the exclusive presence of the sphingomyelin-transfer protein in these tissues [11]. As compared to normal and host liver, a 2–3-fold increase was found in the phosphatidylcholine- and phosphatidylinositol-transfer activities in the fast-growing Morris hepatoma 7777 while these activities were moderately or not increased in the 7787 and 9633 hepatoma [8].

In order to gain insight into the physiological role of the various phospholipid-transfer proteins, sensitive and specific radioimmunoassays are of great value. Recently we described a radioimmunoassay for the phosphatidylcholine-transfer protein from rat liver [17]. This assay was developed for measuring levels of PC-transfer protein in membrane-free cytosols. On the other hand, studies with PC-transfer protein from bovine liver in vitro have shown that this protein tends to interact with membranes [18,19]. It is, therefore, possible that PC-transfer protein in the cell is associated with subcellular organelles. Such associations have been observed for the cellular retinol- and fatty acid-binding proteins in rat [20,21]. In order to determine the levels of PC-transfer protein on membranes we modified our radioimmunoassay, making use of the detergent Triton X-100 [20,22].

In this paper we present data on the subcellular distribution of PC-transfer protein in rat liver and on the levels of PC-transfer protein in the homogenates and 105000 × *g* supernatant fractions from various rat tissues. In addition, levels of PC-transfer protein have been determined in the rat lung during fetal development and in the rat liver after partial hepatectomy.

Materials and Methods

Materials

Carrier-free Na^{125}I was obtained from The Radiochemical Centre (Amersham, U.K.); *Staphylococcus aureus* cells (Pansorbin) from Calbiochem (San Diego, CA, U.S.A.); lactoperoxidase-glucose oxidase 'enzymobeads' from Bio-Rad Laboratories (Richmond, CA, U.S.A.); 1,3,4,6-tetrachloro-3α,6α-diphenyl glycoluril (Iodogen) from Pierce (Rockford, IL, U.S.A.); aprotinin from Boehringer (Mannheim, F.R.G.) and bovine serum albumin (RIA grade) from Sigma (St. Louis, MO, U.S.A.). All other chemicals used were of analytical grade.

Preparation of tissue homogenates and subcellular fractions

Male Wistar rats were fasted overnight. The rats were killed by cervical dislocation and the organs removed, minced and washed with cold 0.25 M sucrose/0.01 M Tris-HCl (pH 7.4) 0.001 M EDTA (buffer 1). A 20% (w/v) homogenate was prepared in this buffer with a Potter-Elvehjem homogenizer. In case of the intestinal mucosa the small intestines were rinsed with ice-cold 0.9% (w/v) NaCl solution, prior to removal of the mucosa and homogenization.

Subcellular fractions were obtained from a 10% (w/v) rat liver homogenate by differential centrifugation; nuclei and cell debris (5 min at 1000 × *g*), heavy mitochondria (10 min at 9000 × *g*), composite or light mitochondria (10 min at 20000 × *g*), microsomes (60 min at 105000 × *g*) and the membrane-free cytosol. These fractions were used without further purification. The pH 5.1 supernatants were prepared from the 105000 × *g* supernatant fractions as described in Ref. 15. Prior to radioimmunoassay, the homogenates and subcellular membrane fractions were solubilized with Triton X-100 (final concentration of 1%, v/v) for 10 min at 0°C; non-solubilized material was removed by centrifugation for 10 min at 14000 × *g*. Protein concentrations were measured by using the method of Lowry et al. [23] with bovine serum albumin as standard.

Purification of PC-transfer protein and immunoglobulin

The purification of PC-transfer protein from rat liver has been described before [8]. The production of antisera in rabbits and the purification of specific IgG by affinity chromatography on PC-transfer protein-Sepharose 4B columns have also been described [8,17].

Radioiodination procedure

PC-transfer protein was radioiodinated with lactoperoxidase-glucose oxidase 'enzymobeads' as described in Ref. 17 or with iodogen as described in Ref. 24. In both cases the ^{125}I-labeled PC-transfer protein was purified by affinity chromatogra-

phy on an anti PC-transfer protein-IgG Sepharose 4B column [17]. Both labeling methods yielded a tracer of comparable quality.

Radioimmunoassay procedure

The buffer used consisted of 0.05 M sodium phosphate (pH 7.2), 1% (v/v) Triton X-100, 0.5% (w/v) bovine serum albumin and 0.02% (w/v) sodium azide. This assay buffer was used to make all dilutions. To 3-ml polystyrene tubes were added: (a) 0.2 ml of sample to be assayed (standard PC-transfer protein solution, tissue homogenate or subcellular fraction). (b) 0.1 ml specific anti-PC-transfer protein-IgG, diluted with assay buffer to give 30–40% binding of ^{125}I-labeled PC-transfer protein in the absence of unlabeled PC-transfer protein. (c) 0.1 ml ^{125}I-labeled PC-transfer protein diluted with assay buffer supplemented with aprotinin (2000 kallikrein inactivator units/ml).

After mixing the contents of the tubes incubation was performed at 4°C for 16–48 h. Antibody-bound ^{125}I-labeled PC-transfer protein was precipitated with the aid of *S. aureus* cells. These cells were washed three times just before use by suspending them in assay buffer and centrifuging for 10 min at 14000 × *g*. After the final washing step, a 1% (w/v) suspension in assay buffer was made and 0.05 ml of this suspension was added to the assay tubes. After incubation for 15–30 min at room temperature, 2 ml of assay buffer was added and the tubes were centrifuged for 10 min at 3000 × *g*. The supernatant was decanted and the radioactivity in the pellet determined by counting in a Packard autogammacounter. Counts bound were expressed as a percentage of the total radioactivity added and plotted against the amount of unlabeled PC-transfer protein. The standard curve was determined in duplicate; samples were assayed at six different dilutions.

Assay of phosphatidylcholine transfer

Transfer of phosphatidylcholine in a microsome-liposome assay was measured as described previously [3], except that incubation was performed for 30 min at 37°C.

Partial hepatectomy

65–75% of the whole liver was removed by the technique described by Higgins and Anderson [25]. The first day after surgery, the animals had access to a 20% solution of dextrose. No further post-operative care was given.

Results

Radioimmunoassay

Fig. 1 shows a standard curve of the radioimmunoassay in combination with a displacement curve for a rat liver 105000 × *g* supernatant fraction. The characteristics of this assay have been described in a previous publication [17]. It measures PC-transfer protein in the range of 5–50 ng and shows no cross-reactivity with other rat liver phospholipid-transfer proteins. In addition the assay cannot be used to measure levels of PC-transfer protein in bovine and mouse tissues. Some modifications with respect to the original procedure were made. Separation of bound from free ^{125}I-labeled PC-transfer protein was accomplished by incubation with *S. aureus* cells instead of goat

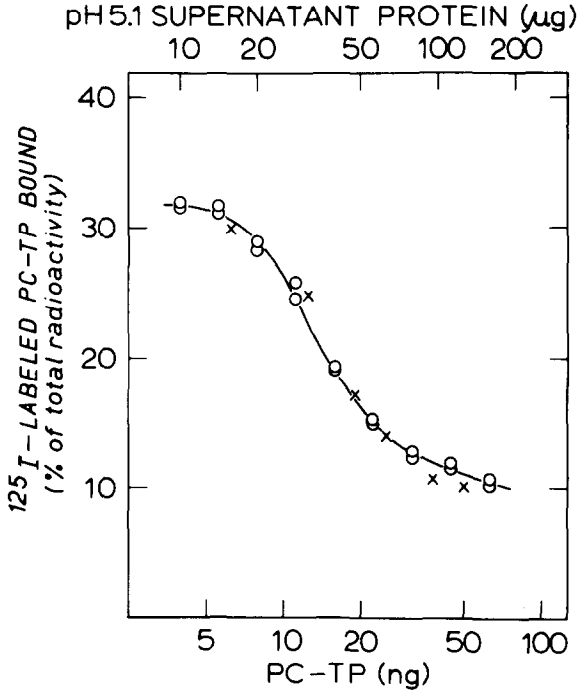

Fig. 1. Radioimmunoassay standard curve for rat liver phosphatidylcholine-transfer protein (PC-TP; ○) and the displacement of antibody-bound ^{125}I-labeled PC-transfer protein by the pH 5.1 supernatant protein from rat liver (×).

anti-rabbit 'immunobeads'. The protein A molecules on the cell walls of these bacteria have a very high affinity for immunoglobulin G, and the high binding capacity and good sedimentation properties make these bacteria a very useful immunoadsorbent [26–28]. Furthermore, a detergent (i.e., Triton X-100) was routinely included in the assay buffer in order to be able to determine PC-transfer protein bound to membrane fractions. Detergents have also been used in radioimmunoassays of, for example, apolipoproteins A-I and C-II in plasma [29,30] and cellular retinol-binding proteins [20,22]. In the presence of Triton X-100, all supernatants, homogenates and subcellular membrane fractions assayed showed displacement curves parallel to the standard curve (see Fig. 1). However, it should be noted that the immunoreactivity of PC-transfer protein in the supernatants was not increased by the detergent, as was observed for the cellular retinol-binding protein [22].

Originally, levels of PC-transfer protein in the supernatants were determined in the absence of proteolytic enzyme inhibitors. Omission of such inhibitors gave erroneous results in the case of the intestinal mucosa. Correct data were obtained by addition of trypsin inhibitor or aprotinin, a universal inhibitor of proteolytic activity [31]. In order to prevent any interference of proteolytic activity in the radioimmunoassay aprotinin was routinely added.

Subcellular distribution of PC-transfer protein

A 10% (w/v) homogenate of rat liver in buffer 1 was fractionated by differential centrifugation and the PC-transfer protein level of the subcellular fractions was determined. Fig. 2 shows the subcellular distribution, expressed as relative specific activity versus percent protein, as suggested by De Duve et al. [32]. The supernatant fraction has the highest specific activity and contains 60% of the total PC-transfer protein, but considerable amounts are present in the other fractions as well. When the mitochondrial or microsomal fraction was washed by resuspension in buffer 1, 20–30% of the PC-transfer protein originally present in these fractions was recovered in the pellet. Apparently, PC-transfer protein is not bound very tightly to these membranes. That there exists an equilibrium between membrane-bound and soluble PC-transfer protein is also suggested by the fact that in 20% (w/v) homogenate only 45% of the transfer protein was found in the supernatant fraction.

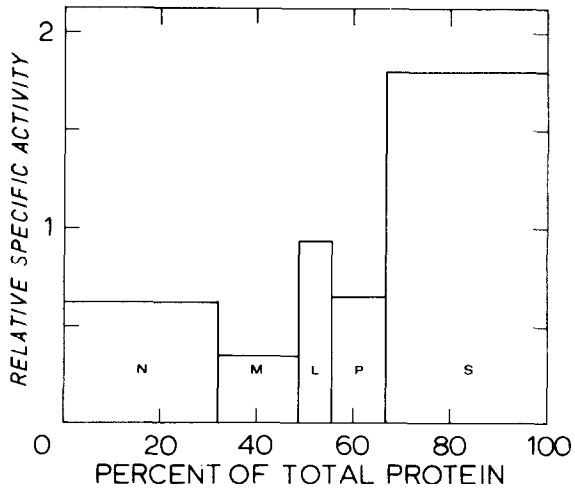

Fig. 2. Subcellular distribution of phosphatidylcholine-transfer protein in rat liver. On the ordinate, each fraction is represented by its relative specific activity (i.e., percentage of recovered PC-transfer protein/percentage of total protein). On the abscissae the protein content of each fraction is expressed as percentage of total recovered protein. Fractions include nuclei and cell debris (N), heavy mitochondria (M), light mitochondria (L), microsomes (P) and supernatant (S).

Levels of PC-transfer protein in different tissues

The concentrations of PC-transfer protein measured in various rat tissues are shown in Table I. Both homogenates and $105000 \times g$ supernatants were assayed. As for the supernatants, the highest values were found in liver and intestinal mucosa in the range of 160–320 ng/mg protein. Kidney, spleen and lung supernatant had intermediate values (30–90 ng/mg protein). Low levels of PC-transfer protein were found in adrenals, whereas brain and heart supernatant did not contain detectable amounts. The same trend was observed when levels of PC-transfer protein were determined in total tissue homogenates, with the highest concentration in liver and the lowest concentration in heart. This indicates that apparently in all tissues studied the fraction of membrane-bound PC-transfer protein is in the same order of magnitude (cf. Fig. 2).

TABLE I

LEVEL OF PHOSPHATIDYLCHOLINE-TRANSFER PROTEIN IN VARIOUS TISSUES

Values represent mean ± S.E. The numbers in parenthesis are the number of samples.

Tissue	PC-transfer protein in 105000 × *g* supernatant (ng/mg protein)	PC-transfer protein in homogenate (μg/g wet weight)
Liver	317±65 (14)	30.3 ±2.0 (9)
Intestinal mucosa	159±16 (7)	not determined
Kidney	92±15 (4)	6.3 ±1.3 (3)
Spleen	87± 8 (4)	7.8 ±0.8 (3)
Lung	33± 4 (6)	1.6 ±0.4 (3)
Adrenals	13 (2)	not determined
Brain	not detected	0.55±0.04 (3)
Heart	not detected	0.42±0.05 (3)

Levels of PC-transfer protein were also determined in regenerating rat liver 2, 4 and 7 days after partial hepatectomy. Despite the rapid growth of the tissue, concentrations did not differ significantly from normal values.

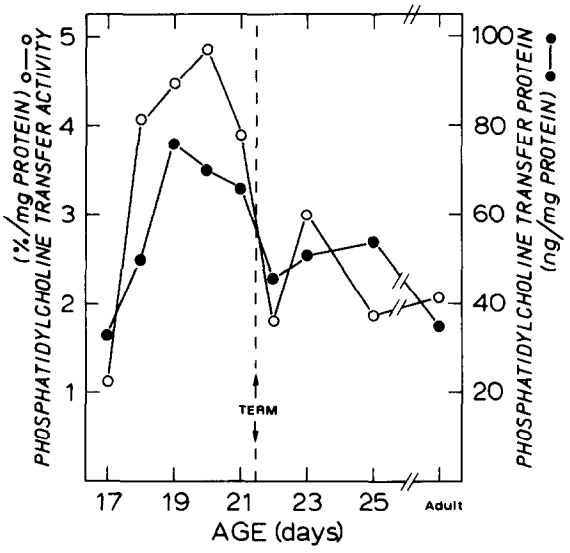

Fig. 3. Level of phosphatidylcholine-transfer protein and phosphatidylcholine-transfer activity in rat lung pH 5.1 supernatant during development. The PC-transfer protein content was determined by radioimmunoassay (●) and the phosphatidylcholine-transfer activity (○) by microsome-vesicle assay as described in Materials and Methods.

Levels of PC-transfer protein during lung development

In a previous study [15] it was found that the phosphatidylcholine transfer activity in the 105000 × *g* supernatant of developing mouse lung changed in parallel with the specific activity of cholinephosphotransferase, the enzyme that catalyzes the last step in the synthesis of phosphatidylcholine. Fig. 3 shows the levels of PC-transfer protein in developing rat lung as measured by radioimmunoassay, together with the phosphatidylcholine-transfer activity. In agreement with developing mouse lung, phosphatidylcholine-transfer activity in rat lung had a maximal value at 2 days before term, which coincided with maximal levels of PC-transfer protein. The sharp rise in levels of PC-transfer protein just before term was followed by a decline which continued until at 3 days post-partem the level of PC-transfer protein approached that of mature lung.

Discussion

This paper describes the use of a specific radioimmunoassay for the measurement of levels of immunoreactive PC-transfer protein in rat tissue homogenates and 105000 × *g* supernatants. In the presence of a detergent (i.e., Triton X-100) tissue homogenates, subcellular fractions and 105000 × *g* supernatants gave rise to displacement curves parallel to the standard curve. The detergent was added to release any PC-transfer protein that was bound to membranes or part of lipid-protein complexes. However, it should be noted that the presence of detergent had no effect on the detectable levels of PC-transfer protein in the 105000 × *g* supernatant from rat liver. Conversely, determination of the cellular retinol-binding protein in rat liver cytosol by radioimmunoassay required that a detergent be present to release this protein from a high-molecular weight lipid-protein aggregate in an immunoreactive form [22].

The determinations of the subcellular distribution of PC-transfer protein in rat liver (Fig. 2) showed that 40% of this protein is associated with particular fractions in 10% (w/v) homogenates. In 20% (w/v) homogenates this percentage is increased to about 60%, suggesting that the fraction that is membrane-bound depends on the actual

membrane concentration. This could mean that in the living cell, where the membrane concentration is much higher than in our experiments, a considerable fraction of the transfer protein is attached to membranes. Two other cellular binding proteins, i.e., the fatty acid-binding protein from rat intestinal mucosa and the cellular retinol-binding protein, have also been shown to be partly associated with particulate fractions [20,21].

As shown in Table I, liver contains the highest amount of PC-transfer protein, followed by the intestinal mucosa, kidney and spleen. This tissue distribution shows a remarkable resemblance with the distribution of two other cellular binding proteins, i.e., the retinol-binding protein [22] and ligandin [33], both of which are present most prominently in liver, kidney and small intestine. Moreover, all three proteins are present at very low levels in brain and heart.

Previously, PC-transfer protein has been partially purified from rat small intestine [34,35] and accounts for approximately 35% of the total phosphatidylcholine-transfer activity [36]. Investigation of the anatomic distribution has indicated that the mucosa has a 4-fold higher phosphatidylcholine-transfer activity per mg of soluble protein than the intestinal wall [34]. Our data show that the levels of PC-transfer protein in rat heart and brain are very low compared to liver. This is in agreement with immunological studies on levels of PC-transfer protein in bovine tissues [6]. It is of interest to note that the $105000 \times g$ supernatant from rat brain does not have any phosphatidylethanolamine-transfer activity [37]. This implies that rat brain lacks the non-specific phospholipid-transfer protein which presumably is responsible for this activity [7]. Its absence, in conjunction with the low levels of PC-transfer protein, shows that virtually all phospholipid-transfer activity in rat brain is due to the phosphatidylinositol-transfer protein [6,37].

Phospholipid-transfer proteins are thought to be involved in the secretion of lung surfactant, in which process the alveolar type II cells supposedly play a central role [38,39]. Recent studies have indicated that these cells from adult rat lung contain only the non-specific phospholipid-transfer protein, arguing against PC-transfer protein being involved in the production and secretion of lung surfactant [40]. On the other hand, levels of PC-transfer protein in lung are maximal just before term, at which time the production of lung surfactant is at its highest rate (Fig. 3). Concomitant with the increase of the PC-transfer protein concentration, an increase in the activities of choline kinase and cholinephosphotransferase was observed [15,41]. The significance of this correlation remains to be established.

PC-transfer protein is thought to be involved in the intracellular transport of phosphatidylcholine from the site of synthesis, i.e., the endoplasmic reticulum, to those sites where phosphatidylcholine is required [37]. In this respect it is worth noting that of all tissues examined the highest levels of PC-transfer protein are present in liver and intestinal mucosa. It may well be that PC-transfer protein is involved in the very active phosphatidylcholine metabolism of these tissues and plays a role in processes such as the formation and secretion of lipoproteins. Recently, PC-transfer protein from bovine liver has been shown to mediate the net transfer of phosphatidylcholine to membranes deficient in this phospholipid [42], and complexes consisting of sphingomyelin and apolipoprotein A-II [43].

References

1 Kader, J.C. (1977) in Dynamic Aspects of Cell Surface Organization (Poste, G. and Nicolson, G.L., eds.), pp. 127–204, Elsevier/North-Holland Biomedical Press, Amsterdam
2 Wirtz, K.W.A. (1982) in: Lipid-Protein Interactions (Jost, P.C. and Griffith, O.H., eds.), pp. 151–233, Wiley-Interscience, New York
3 Kamp, H.H., Wirtz, K.W.A. and Van Deenen, L.L.M. (1973) Biochim. Biophys. Acta 318, 313–325
4 Crain, R.C. and Zilversmit, D.B. (1980) Biochemistry 19, 1433–1439
5 DiCorleto, P.E., Warach, J.B. and Zilversmit, D.B. (1979) J. Biol. Chem. 254, 7795–7802
6 Helmkamp, G.M., Nelemans, S.A. and Wirtz, K.W.A. (1976) Biochim. Biophys. Acta 424, 168–182
7 Bloj, B. and Zilversmit, D.B. (1977) J. Biol. Chem. 252, 1613–1619
8 Poorthuis, B.J.H.M., Van der Krift, T.P., Teerlink, T., Akeroyd, R., Hostetler, K.Y. and Wirtz, K.W.A. (1980) Biochim. Biophys. Acta 600, 376–386
9 Poorthuis, B.J.H.M., Glatz, J.F.C., Akeroyd, R. and Wirtz, K.W.A. (1981) Biochim. Biophys. Acta 665, 256–261
10 Dyatlovitskaya, E.V., Timofeeva, N.G. and Bergelson, L.D. (1978) Eur. J. Biochem. 82, 463–471

11 Dyatlovitskaya, E.V., Timofeeva, N.G., Yakimenko, E.F., Barsukov, L.I., Muzya, G.I. and Bergelson, L.D. (1982) Eur. J. Biochem. 123, 311–315
12 Douady, D., Grosbois, M., Guerbette, F. and Kader, J.C. (1982) Biochim. Biophys. Acta 710, 143–153
13 Brophy, P.J. and Aitken, J.W. (1979) J.Neurochem. 33, 355–356
14 Ruenwongsa, P., Singh, H. and Jungalwala, F.B. (1979) J. Biol. Chem. 254, 9385–9393
15 Engle, M.J., Van Golde, L.M.G. and Wirtz, K.W.A. (1978) FEBS Lett. 86, 277–281
16 Lumb, R.H., Cottle, D.A., White, L.C., Hoyle, S.N., Pool, G.L. and Brumley, G.W. (1980) Biochim. Biophys. Acta 620, 172–175
17 Teerlink, T., Poorthuis, B.J.H.M., Van der Krift, T.P. and Wirtz, K.W.A. (1981) Biochim. Biophys. Acta 665, 74–80
18 Machida, K. and Ohnishi, S. (1978) Biochim. Biophys. Acta 507, 156–164
19 Wirtz, K.W.A., Vriend, G. and Westerman, J. (1979) Eur. J. Biochem. 94, 215–221
20 Adachi, N., Smith, J.E., Sklan, D. and Goodman, D.S. (1981) J. Biol. Chem. 256, 9471–9476
21 Ockner, R.K. and Manning, J.A. (1974) J. Clin. Invest. 54, 326–338
22 Sklan, D., Blaner, W.S., Adachi, N., Smith, J.E. and Goodman, D.S. (1982) Arch. Biochem. Biophys. 214, 35–44
23 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
24 Salacinski, P.R.P., McLean, C., Sykes, J.E.C., Clement-Jones, V.V. and Lowry, P.J. (1981) Anal. Biochem. 117, 136–146
25 Higgins, G.M. and Anderson, R.M. (1931) Arch. Path. 12, 186–202
26 Kessler, S.W. (1975) J. Immunol. 115, 1617–1624
27 Kessler, S.W. (1976) J. Immunol. 117, 1482–1490
28 O'Keefe, E. and Bennett, V. (1980) J. Biol. Chem. 255, 561–568
29 Mao, S.J.T. and Kottke, B.A. (1980) Biochim. Biophys. Acta 620, 447–453
30 Barr, S.I., Kottke, B.A., Chang, J.Y. and Mao, S.J.T. (1981) Biochim. Biophys. Acta 663, 491–505
31 Zyznar, E.S. (1981) Life Sci. 28, 1861–1866
32 De Duve, C., Pressman, B.C., Gianetto, R., Wattiaux, R. and Appelmans, F. (1955) Biochem. J. 60, 604–617
33 Bass, N.M., Kirsch, R.E., Tuff, S.A. and Saunders, S.J. (1977) Biochim. Biophys. Acta 494, 131–143
34 Lutton, C. and Zilversmit, D.B. (1976) Lipids 11, 16–20
35 Yamada, K., Sasaki, T. and Sakagami, T. (1978) J. Biochem. 84, 855–863
36 Lutton, C. and Zilversmit, D.B. (1976) Biochim. Biophys. Acta 441, 370–379
37 Wirtz, K.W.A. (1974) Biochim. Biophys. Acta 344, 95–117
38 Askin, F.B. and Kuhn, C. (1971) Lab. Invest. 25, 260–268
39 Goerke, J. (1974) Biochim. Biophys. Acta 344, 241–261
40 Post, M., Batenburg, J.J., Schuurmans, E.A.J.M. and Van Golde, L.M.G. (1980) Biochim. Biophys. Acta 620, 317–321
41 Farrell, P.M., Lundgren, D.W. and Adams, A.J. (1974) Biochem. Biophys. Res. Commun. 57, 696–701
42 Wirtz, K.W.A., Devaux, P.F. and Bienvenue, A. (1980) Biochemistry 19, 3395–3399
43 Wilson, D.B., Ellsworth, J.L. and Jackson, R.L. (1980) Biochim. Biophys. Acta 620, 550–561