Journal of Cellular Biochemistry 72:349–355 (1999)

Isolation of Plasma Membranes and Golgi Apparatus From a Single Chicken Liver Homogenate

Lieve Vleurick,[1]* Eduard R. Kühn,[1] Eddy Decuypere,[1] and Paul P. Van Veldhoven[2]

[1]Leuven Poultry Research Group, Zoological Institute and Faculty of Agronomy, B-3000 Leuven, Belgium
[2]Faculty of Medicine, Department of Pharmacology, Katholieke Universiteit Leuven, Belgium

Abstract Chicken liver plasma membranes, minimally contaminated with Golgi apparatus-derived vesicles, were prepared from a low-speed (400*g*) pellet by means of flotation in isotonic Percoll solution, followed by a hypotonic wash and flotation in a discontinuous sucrose gradient. Based on the analysis of suitable marker enzymes, alkaline phosphatase and alkaline phosphodiesterase, two plasma membrane fractions were isolated with enrichments, depending on the equilibrium density and marker of 28–97 and with a total yield of 4–5%. Golgi apparatus fractions were prepared by flotation of microsomes, obtained from the same homogenate as the low-speed pellet, in a discontinuous sucrose gradient. The *trans*-Golgi marker galactosyltransferase was 27-fold enriched in a fraction of intermediate density (d=1.077–1.116 g/ml). Approximately 12% of galactosyltransferase was recovered in the membranes equilibrating d=1.031–1.148 g/ml. Contamination with plasma membrane fragments was low in the light (d=1.031–1.077 g/ml) and intermediate density Golgi vesicles. The isolation of purified plasma membranes and Golgi vesicles from one liver homogenate will enable future studies on receptor cycling between these cell organelles. J. Cell. Biochem. 72:349–355, 1999.

Key words: subcellular fractionation; plasma membranes; Golgi apparatus; marker enzymes; chicken; liver

In chicken, low hepatic growth hormone concentrations coincide with high circulating growth hormone levels [Vanderpooten et al., 1991]. In rats, it was demonstrated that the growth hormone receptor, located in the plasma membrane, is internalized upon ligand binding. The receptors are then partly recycled through the Golgi apparatus to the cell membrane [Hochberg et al., 1993]. To gain a better insight into the internalization, degradation, and recycling of growth hormone receptors in chicken liver, procedures to obtain highly purified plasma membrane and Golgi apparatus fractions from one liver are desirable. The techniques of subcellular fractionation however have been worked out mainly on rodent tissues, especially liver. In the few reports dealing with chicken liver, homogenates for plasma membrane or Golgi vesicle isolation were prepared differently. In addition, enrichments of the final fractions were poorly documented and contamination of the plasma membrane fraction with other organelles was rather high [Gavazova et al., 1980; Banerjee and Redman, 1983].

Another problem relates to the use of marker enzymes, indispensable in order to estimate the enrichments of the isolated subcellular fractions. The list of markers for rat hepatic plasma membrane is long and includes Mg^{2+}-stimulated ATPase, Na^+,K^+,Mg^{2+}-dependent ATPase, 5′-nucleotidase, alkaline phosphodiesterase (APD), alkaline phosphatase (AP) and K^+-activated nitrophenylphosphatase [Emmelot et al., 1964; Beaufay et al., 1974b; Kashiwamata et al., 1979]. Cholesterol and sialic acid are also considered to be plasma membrane specific constituents [Emmelot et al., 1964; Beaufay et al., 1974b]. In general, the use of these markers has not been evaluated in chicken liver.

Because of the biochemical and morphological heterogeneity of the Golgi apparatus, its characterization is even more complex compared with that of plasma membranes. As a marker enzyme for *cis*-Golgi, the side of the Golgi stack next to the nucleus and the transitional endoplasmic reticulum, α-mannosidase has been used [Tabas and Kornfeld, 1979], whereas galactosyltransferase (GT) is charac-

*Correspondence to: Lieve Vleurick, Unité de Biologie Moléculaire et Physiologie Animale, Faculté Universitaire des Sciences Agronomiques, 13 Avenue Maréchal Juin, B-5030 Belgium. E-mail: vleurick.l@fsagx.ac.be

Received 16 July 1998; Accepted 11 August 1998

teristic for *trans*-located Golgi elements [Bergeron et al., 1973; Beaufay et al., 1974b].

In this paper, we present a method that allows isolation of chicken hepatic plasma membrane and Golgi apparatus fractions from a single homogenate. An optimal combination of high yield and low mutual contamination of these fractions was aimed. The Percoll flotation step followed by a hypotonic wash provides a simple method to prepare crude plasma membrane fractions and can also be applied to other species, as documented in a preliminary form for rat liver [De Ceuster et al., 1995]. The rat markers mentioned above were assayed in liver homogenate or subcellular fractions, or both, in order to find reliable plasma membrane and Golgi markers with which to assess enrichments for these organelles and to optimize the different centrifugation steps.

MATERIALS AND METHODS

Materials

Common laboratory chemicals were from local suppliers. Percoll was obtained from Pharmacia (Uppsala, Sweden). Tetramisole was purchased from Janssen Chimica (Geel, Belgium). UDP-[^{3}H]galactose was labeled by Amersham (Bucks, UK). Enzyme substrates (IMP, p-nitrophenylphosphate, p-nitrophenylthymidine-5-monophosphate, UDP-galactose) and Triton-X-100 were from Boehringer Mannheim (Mannheim, Germany).

Differential Centrifugation

Adult brown laying hens (Hisex, Euribrid, Boxmeer, The Netherlands) in their first laying year were housed in battery cages with free access to water and feed. Chickens were killed by decapitation and livers were quickly excised and immersed in ice-cold homogenization medium (ST: 0.25 M sucrose and 5 mM Tris-HCl pH 7.5). During all subsequent steps, sample temperature was kept at 0–4°C, and solutions and containers were precooled on ice. To a 40-ml Dounce homogenizer, 5 g of minced liver was transferred, about 30 ml of ST was added, and the tissue was ground by hand with 10 strokes of a loose-fitting pestle. All liver homogenate was combined and brought with ST to 10 ml/g of initial liver weight (25–35 g total). The homogenate was subsequently subjected to differential centrifugation. In a first centrifugation whole cells and large cell fragments were precipitated at low speed (400*g*, 12 min). The resulting pellet consisted of three layers: a small bicolor red-topped brown pellet, presumably nonhomogenized liver and red blood cells, a brown jelly-like layer, and a drab loose top layer. The supernatant was slowly decanted, without disturbing the loose top layer. The pellet was resuspended and washed twice with respectively 5 and 4 ml of ST per g liver. The final pellet (corresponding to a nuclear fraction and named N) was resuspended in 1 ml of ST per g liver. The combined postnuclear supernatants (E) were submitted to successive increasing *g* forces (1,500*g* for 10 min, 10,000*g* for 20 min, and 100,000*g* for 1 h, 5 min). The fractions obtained were named M, L, P, and S and were enriched, respectively, in mitochondria, lysosomes and peroxisomes, microsomes, and cytosol.

Plasma Membrane

In order to obtain purified plasma membranes, the 400*g* pellet N was mixed with an equal volume of isotonic Percoll solution to obtain final concentrations of 20% (w/v) Percoll, 0.25 mM EDTA.Na_2, 0.5 mM 3-[N-morpholino]propanesulfonic acid (Mops), 0.25 M sucrose, and 2.5 mM Tris-HCl pH 7.5. This suspension was spun at low speed (Sorvall SS-34 rotor, 1,500*g*, 15 min). At the end of the run, a bilayered pellet was visible consisting of a small red pellet covered by a larger brown layer. A jelly-like fibrous reddish layer was floating on the cloudy ochre Percoll solution. These floating membranes were collected with a Pasteur pipette and diluted to 10 ml per g liver with a hypotonic solution (1 mM $NaHCO_3$ pH 7.5) to disrupt membrane-surrounded organelles. The membranes were then pelleted in an ultracentrifuge (100,000*g*, 1 h 5 min). To reduce the final volume further, the resulting pellets were combined and centrifuged again. This hypotonic pellet was resuspended with heavy sucrose solution (d=1.298 g/ml), to obtain a 1.5 M sucrose concentration (1 ml per g liver). All sucrose solutions contained 5 mM Tris-HCl pH 7.5. Density was checked with a hand refractometer (η = 1.406). A discontinuous sucrose gradient was constructed in a 14-ml polycarbonate ultracentrifuge tube, as follows. The crude plasma membrane fraction (4 ml) was overlaid with 4 ml 1.3 M, 4 ml 0.9 M, and 1 ml 0.25 M sucrose, and centrifuged for 1 h, 35 min at 100,000*g* in a Beckman SW40Ti swing-out rotor. The white

bands formed at the upper two interphases and the remaining solution were collected, diluted to 0.25 M sucrose, precipitated at 100,000*g* for 35 min, and resuspended in a small volume of ST.

Golgi Vesicles

Golgi-derived vesicles were isolated by the method of Ehrenreich et al. [1973]. Briefly, the microsomal fraction was brought to 1.3 M sucrose with heavy sucrose (d=1.298 g/ml), 4 ml of which (1 ml per g liver) was used as cushion of a discontinuous sucrose gradient and overlaid with 3 ml 1.15 M, 2 ml 0.9 M, 2 ml 0.6 M, and 2 ml 0.25 M sucrose. After centrifugation for 1 h, 35 min at 100,000*g* in a Beckman SW40Ti rotor, fractions were collected from the different interphases and processed as described above for the plasma membrane gradient.

Marker Enzymes and Chemical Constituents

At each step, aliquots were taken from every fraction obtained and stored at −20°C until protein and marker enzyme analysis. Protein determinations were as described by Peterson [1977]. GT was assayed by measuring the transfer of tritiated galactose (1 mCi/mmol) from an UDP carrier to ovalbumin, according to Van Veldhoven and Mannaerts [1991], with the following modifications. Reaction volume was reduced to 50 µl. After 1-h incubation at 30°C, samples were stopped with 20 µl 0.3 M EDTA.Na_4; 50-µl aliquots were spotted on a Whatman GF/C filter. Filters were washed in trichloroacetic acid (TCA) and ether, precipitated proteins were dissolved in 0.5 ml 1 N NaOH and lysates were neutralized with 0.1 ml 5 N acetic acid to minimize chemoilluminescence. α-Mannosidase, with α-nitrophenylmannoside as substrate, was determined at pH 5.5, the optimal pH for the Golgi enzyme [Dewald and Touster, 1973].

For 5′-nucleotidase measurements, the hydrolysis of 5′-IMP was followed at pH 6.5 [Itoh et al., 1967]. Reactions contained enzyme, 4 mM 5′-IMP, 10 mM $MgCl_2$, 10 mM L-tartrate and 50 mM piperazine-N,N′-bis[2-ethanesulfonic acid] (Pipes)-NaOH buffer pH 6.5 in a final volume of 0.25 ml. Tubes were incubated at 37°C for 20 min, stopped with 1 ml 10% (w/v) TCA, and centrifuged. Phosphate concentrations were determined in 0.5 ml supernatant [Van Veldhoven et al., 1991]. Aspecific phosphate production was corrected for by omitting $MgCl_2$ from the reaction mixture. AP and APD assays were adapted from Beaufay et al. [1974a] and are based on the rate of formation of p-nitrophenol using, respectively, p-nitrophenylphosphate and p-nitrophenylthymidinephosphate as substrates. Concentrations for APD were as described. Final concentrations in AP assay were 2 mM substrate, 2 mM $MgCl_2$, 20 µM Zn-acetate, 0.1% (v/v) Triton X-100, and 50 mM glycine-NaOH pH 10.5 in a volume of 0.5 ml. Reaction was started by substrate addition after preincubation of reaction mixture for 5–10 min. The kinetics of substrate hydrolysis at 37°C were calculated from absorption data (at 420 nm) and corrected for chemical hydrolysis of p-nitrophenylphosphate.

RESULTS AND DISCUSSION

Several protocols, described for the analysis of plasma membrane and Golgi markers in rat liver, were tested in chicken liver fractions. Despite attempts to adjust assay conditions only three enzymes, AP and APD for plasma membrane and GT for the Golgi apparatus, appeared suitable for chicken liver. Their activities and recovery after subcellular fractionation are shown in Table I. AP, APD, and GT activities were of the same order of magnitude as reported by Amar-Costesec et al. [1974] for rats. AP was not affected by the mammalian inhibitor tetramisole [Van Belle, 1972].

Other rat liver plasma membrane marker assays could not be used in chickens. In agreement with Itoh et al. [1967], hydrolysis of 5′-IMP, a proposed 5′-nucleotidase substrate, was higher than that of 5′-AMP in chicken liver. The activity with 5′-IMP amounted to 1.1 U/g, still 10 times lower than 5′-AMP nucleotidase activity in rats [Van Veldhoven and Mannaerts, 1991; Amar-Costesec et al., 1974]. Moreover, three-fourths of activity was found in the cytosolic fraction. The high cytosolic activity is most likely due to soluble aspecific phosphatases, and not to 5′-nucleotidase released from the plasma membrane. The addition of Ni^{2+} or concanavalin, reported inhibitors of 5′-nucleotidase in rat liver [Itoh et al., 1967; Riordan et al., 1974], did not, or did not completely, reduce 5′-IMP hydrolysis, also pointing to a nonspecific phosphatase. The Na^+,K^+-ATPase [Penefsky and Bruist, 1984] and K^+-sensitive nitrophenylphosphatase [Kashiwamata et al., 1979] assays were hindered by a largely aspecific

TABLE I. Protein Content and Marker Enzymatic Activities in Chicken Liver Homogenate[a]

	No. of determinations	Activity or amount per g liver[b]	Spec act (mU/mg protein)	Recovery[c] (%)
Protein (mg)	6	155.2 ± 3.4	—	93.8 ± 5.9
GT (mU)	4	21.5 ± 1.4	0.139 ± 0.007	62.0 ± 3.2
APD (U)	6	7.13 ± 0.94	46.3 ± 7.2	92.4 ± 5.1
AP (U)	6	4.27 ± 0.73	27.5 ± 4.6	75.2 ± 4.3

[a]Data are means ±SEM.
[b]Combined sum of activity or amount present in the nuclear fraction (400*g* pellet) and postnuclear supernatant. Units (U) are μmol produced per min.
[c]Recovery was calculated as the percentage of the sum of activities or amounts present in all fractions obtained by differential centrifugation divided by those found in nuclear fraction and postnuclear supernatant ((N + M + L + P + S)/(N + E)).

substrate hydrolysis. Sialic acid measurements, based on determination of released N-acetylglucosamine [Jourdian et al., 1971], were complicated by the presence of other absorbing substances (data not shown).

After differential centrifugation, analysis of the plasma membrane and Golgi markers in chicken hepatic subcellular fractions showed distributions resembling those seen in rat liver. GT was mainly recovered in the microsomal fraction (50% when corrected for low GT recovery). APD and AP showed the typical nucleomicrosomal pattern. A gentle homogenization disrupts the cell membrane only at certain places to yield large membrane sheets consisting of adhering plasma membranes from two neighboring cells [Neville et al., 1960; Emmelot et al., 1964; Hubbard et al., 1983]. These sheets precipitate at low *g*-forces together with the nuclei and whole cells. Small plasma membrane pieces round up and become part of the microsomal fraction.

Table II presents marker enzyme analysis throughout plasma membrane purification. Because of the more favorable plasma membrane marker to GT ratio, the 400*g* pellet was used as start material for isolation of plasma membrane fragments. Membranes enriched in plasma membrane markers floated to the higher interphases of a discontinuous sucrose gradient. The enrichment of AP and APD was excellent (28- to 52-fold for the 0.9/1.3 M interphase and 95- to 97-fold for the 0.25/0.9 M interphase, respectively), certainly when compared with the work of Gavazova et al. [1980], whose chicken hepatic plasma membranes were enriched in plasma membrane markers only 1.5–5 times. Jelsema and Morré [1978] substantiated their report on rat liver fractionation with morphometric data and marker enzyme analysis and calculated that 4% of total liver proteins was plasma membrane derived. Hubbard et al. [1983] found a 40-fold enrichment in plasma membrane markers for a plasma membrane preparation that contained all subdomains. Hepatocytes have a sinusoidal front, lateral surfaces, and a bile canalicular front. Compared with these reports, our enrichments of the light plasma membrane fraction are high and may indicate that chicken hepatocytes contain relatively less plasma membrane or that a specific plasma membrane subdomain is isolated.

Microsomal fractions, enriched 3.5 times in GT, somewhat less in AP and APD, were chosen as start material for the isolation of Golgi-derived vesicles. Table III illustrates marker enzyme enrichment throughout Golgi vesicle isolation. For our experiments, the method of Ehrenreich et al. [1973] based on discontinuous sucrose gradients was followed, and we isolated a so-called heavy, an intermediate and a light Golgi fraction at interphases corresponding to densities of 1.148–1.116, 1.116–1.077, and 1.077–1.031 g/ml. Our Golgi fractions were 8–27 times enriched in GT, similar to other chicken hepatic preparations [Banerjee and Redman, 1984; Gavazova et al., 1986]. Approximately 12% of GT was recovered in the three Golgi fractions. The highest GT specific activity was found in the intermediate fraction, which differs from previous results [Gavazova et al., 1986]. In the literature, morphological (based on the presence of very low density lipoprotein-filled particles) and biochemical (based on marker enzyme analysis) characteristics of Golgi fractions sometimes deviate. Generally, the density of Golgi vesicles decreases in the order of *cis*, median, *trans*. The light fraction is the purest and has the most *trans*-like appearance from a morphological point of view [Ehrenreich et al., 1973]. However, although GT is widely used as a *trans* marker, in some publica-

TABLE II. Presence of Marker Enzymes at Different Steps in the Plasma Membrane Purification Procedure[a]

	% protein	% GT	SA[b] GT (mU/mg protein)	% APD	SA[b] APD (mU/mg protein)	% AP	SA[b] AP (mU/mg protein)
400*g* pellet (N)	17.0 ± 2.8 (6)	2.76 ± 1.15 (4)	0.017 ± 0.004	25.7 ± 2.3 (6)	72.4 ± 10.5	22.2 ± 3.4 (6)	39.5 ± 10.2
Percoll flotation	6.1 ± 1.1 (5)	1.65 ± 0.79 (3)	0.028 ± 0.014	29.4 ± 4.6[c] (5)	238 ± 34	11.7 ± 1.0 (5)	66.2 ± 24.9
Hypotonic pellet	3.8 ± 0.8 (5)	1.29 ± 0.65 (4)	0.038 ± 0.015	19.9 ± 3.7 (5)	297 ± 42	16.8 ± 2.7 (5)	161.9 ± 47.4
Light plasma membranes[d]	0.018 ± 0.004 (4)	0.085 ± 0.049 (4)	0.55 ± 0.26	1.37 ± 0.43 (4)	4511 ± 1160	2.23 ± 0.81 (4)	2602 ± 663
Heavy plasma membranes	0.073 ± 0.020 (4)	0.28 ± 0.16 (4)	0.43 ± 0.14	2.95 ± 0.86 (4)	2402 ± 798	2.99 ± 1.60 (4)	762 ± 145

[a]Data are means ±SEM. Units (U) are µmol produced per min. Number of fractionations is given between parentheses. Protein and enzyme activities are expressed as a percentage of nuclear fraction and postnuclear supernatant. Recoveries for protein and enzymes were 70–125% in the final gradient.
[b]Specific activity.
[c]For unknown reasons, more activity is recovered than present in the previous step.
[d]Light and heavy plasma membranes were recovered from respectively the 0.25/0.9 and 0.9/1.3 M sucrose interphases of the final gradient.

TABLE III. Presence of Marker Enzymes at Different Steps in Golgi Vesicle Isolation Procedure[a]

	% protein	% GT	SA[b] GT (mU/mg protein)	% APD	SA[b] APD (mU/mg protein)	% AP	SA[b] AP (mU/mg protein)
Microsomal fraction (P)	8.45 ± 0.47	30.3 ± 2.9	0.48 ± 0.05	28.5 ± 3.1	169 ± 33	19.7 ± 2.7	54.7 ± 8.7
Light Golgi vesicles 0.25/0.6 M	0.19 ± 0.04	1.7 ± 0.3	1.10 ± 0.14	0.05 ± 0.02	13.7 ± 3.9	0.27 ± 0.12	28.7 ± 9.3
Intermediate Golgi vesicles 0.6/0.9 M	0.21 ± 0.03	6.2 ± 0.5	3.80 ± 0.55	0.26 ± 0.03	61.9 ± 7.0	0.36 ± 0.07	39.9 ± 6.7
Heavy Golgi vesicles 0.9/1.15 M	0.23 ± 0.04	4.6 ± 0.7	2.36 ± 0.21	3.3 ± 0.8	662 ± 104	2.8 ± 0.6	275 ± 62
Interphase 1.15/1.3 M	0.59 ± 0.06	2.7 ± 0.1	0.60 ± 0.03	7.6 ± 1.9	589 ± 107	5.2 ± 1.1	192 ± 31

[a]Data are means ±SEM. Protein, APD, and AP measurements were based on five experiments, GT was determined on four gradients. Units (U) are µmol produced per min. Protein amounts and enzyme activities are expressed as a percentage of nuclear fraction and postnuclear supernatant. Recovery in the final gradient, expressed relative to the microsomal fraction, amounted to 52%, 74%, 69%, and 87% for protein, GT, APD, and AP, respectively. Presumably, during pelleting of the diluted gradient fractions, some loss of material occurred, explaining the low recoveries.
[b]Specific activity.

tions the highest GT specific activities are present in the heavy fractions [Bergeron et al., 1973], whereas in others morphological and biochemical data correspond [Bergeron, 1979].

To check the validity of GT as Golgi marker and to correct for *cis-trans* variations, a *cis*-Golgi marker, α-mannosidase, was tested [Dewald and Touster, 1973]. Because of the nonhomogeneous Golgi localization of GT, Golgi content of light Golgi fractions might be underestimated. In chicken liver however, α-mannosidase activity was not only associated with Golgi. Substantial activity was seen in the L fraction, presumably due to the low assay pH and reflecting lysosomal contribution (L: 72.4 mU/g; P: 17.8 mU/g). The distribution of α-mannosidase in the Golgi gradient was similar to that of GT (specific activities in mU/mg protein): 0.25/0.6 M 3.3, 0.6/0.9 M 5.9, 0.9/1.15 M 3.6, and 1.15/1.3 M 2.0) and counterindicates for an increase in *trans* elements with increasing density.

In conclusion, for the first time Golgi and plasma membrane fractions were isolated from a single chicken liver homogenate, enabling future studies on receptor trafficking between these organelles. A low mutual contamination was achieved compared with previous studies, using a high low-speed pellet for the isolation of Golgi vesicles and plasma membranes, respectively. Purity of plasma membrane fraction was further improved by a novel Percoll flotation step.

REFERENCES

Amar-Costesec A, Beaufay H, Wibo M, Thinès-Sempoux D, Feytmans E, Robbi M, Berthet J. 1974. Analytical study of microsomes and isolated subcellular membranes from rat liver. II. Preparation and composition of the microsomal fraction. J Cell Biol 61:201–212.

Banerjee D, Redman CM. 1983. Biosynthesis of high-density lipoprotein by chicken liver. Nature of nascent intracellular high density lipoprotein. J Cell Biol 96:651–660.

Banerjee D, Redman, CM. 1984. Biosynthesis of high-density lipoprotein by chicken liver. Conjugation of nascent lipids with apoprotein A1. J Cell Biol 99:1917–1926.

Beaufay H, Amar-Costesec A, Feytmans E, Thinès-Sempoux D, Wibo M, Robbi M, Berthet J. 1974a. Analytical study of microsomes and isolated subcellular membranes from rat liver. I. Biochemical methods. J Cell Biol 61:188–200.

Beaufay H, Amar-Costesec A, Thinès-Sempoux D, Wibo M, Robbi M, Berthet J. 1974b. Analytical study of microsomes and isolated subcellular membranes from rat liver. III. Subfractionation of the microsomal fraction by isopycnic and differential centrifugation in density gradients. J Cell Biol 61:213–231.

Bergeron JJM. 1979. Golgi fractions from livers of control and ethanol-intoxicated rats. Enzymatic and morphologic properties following rapid isolation. Biochim Biophys Acta 555:493–503.

Bergeron JJM, Ehrenreich JH, Siekevitz P, Palade GE. 1973. Golgi fractions prepared from rat liver homogenates. II. Biochemical characterization. J Cell Biol 59: 73–88.

De Ceuster P, Mannaerts GP, Van Veldhoven PP. 1995. Identification and subcellular localization of spinganine-phosphatases in rat liver. Biochem J 311:139–146.

Dewald B, Touster O. 1973. A new α-D-mannosidase occurring in Golgi membranes. J Biol Chem 248:7223–7233.

Ehrenreich JH, Bergeron JJM, Siekevitz P, Palade GE. 1973. Golgi fractions prepared from rat liver homogenates. I. Isolation procedure and morphological characterization. J Cell Biol 59:45–72.

Emmelot P, Bos CJ, Benedetti EL, Rümke Ph. 1964. Studies on plasma membranes. I. Chemical composition and enzyme content of plasma membranes isolated from rat liver. Biochim Biophys Acta 90:126–145.

Gavazova E, Ivanov S, Chelibonova-Lorer H. 1980. Isolation and partial characterization of plasma membranes from chicken liver and from Mc-29 virus induced transplantable hepatoma. Neoplasma 27:399–408.

Gavazova E, Ivanov S, Chelibonova-Lorer H. 1986. Isolation and characterization of chicken liver and hepatoma Mc-29 endoplasmic reticulum and Golgi apparatus membranes and biosynthesis of their glycoconjugates. J Natl Cancer Inst 78:683–693.

Hochberg Z, Phillip M, Youdim MBH, Amit T. 1993. Regulation of the growth hormone (GH) receptor and GH-binding protein by GH pulsatility. Metabolism 142:1617–1623.

Hubbard AL, Wall DA, Ma A. 1983. Isolation of rat hepatocyte plasma membranes. I. Presence of the three major domains. J Cell Biol 96:217–229.

Itoh R, Mitsui A, Tsushima K. 1967. 5′-nucleotidase of chicken liver. Biochim Biophys Acta 146:151–159.

Jelsema CL, Morré DJ. 1978. Distribution of phospholipid biosynthetic enzymes among cell components of rat liver. J Biol Chem 253:7960–7971.

Jourdian GW, Dean L, Roseman S. 1971. The sialic acids. XI. A periodate-resorcinol method for the quantitative estimation of free sialic acids and their glycosides. J Biol Chem 246:430–435.

Kashiwamata S, Goto S, Semba RK, Suzuki FN. 1979. Inhibition by bilirubin of $(Na^+ + K^+)$-activated adenosine triphosphatase and K^+-activated p-nitrophenylphosphatase activities of NaI-treated microsomes from young rat cerebrum. J Biol Chem 254:4577–4584.

Neville DM Jr. 1960. The isolation of a cell membrane fraction from rat liver. J Biophys Biochem Cyt 8:413–422.

Penefsky HS, Bruist MF. 1984. Adenosinetriphosphatases. In: Bergmeyer HU, editor. Methods of enzymatic analysis. IV. Enzymes 2: Esterases, glycosidases, lyases, ligases. Weinheim: Verlag Chemie. p 324–335.

Peterson GL. 1977. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal Biochem 83:346–356.

Riordan JR, Slavik M. 1974. Interactions of lectins with membrane glycoproteins. Effects of concanavalin A on 5′-nucleotidase. Biochim Biophys Acta 373:356–360.

Tabas I, Kornfeld S. 1979. Purification and characterization of a rat liver Golgi α-mannosidase capable of processing asparagine-linked oligosaccharides. J Biol Chem 254: 11655–11663.

Va Belle H. 1972. Kinetics and inhibtion of alkaline phosphatases from canine tissues. Biochim Biophys Acta 289:158–168.

Vanderpooten A, Darras VM, Hyubrechts LM, Rudas P, Decuypere E, Kühn ER. 1991. Effect of hypophysectomy and acute administration of growth hormone (GH) on GH-receptor binding in chick liver membranes. J Endocrinol 129:275–281.

Van Veldhoven PP, Mannaerts GP. 1991. Subcellular localization and membrane topology of sphingosine-1-phosphate lyase in rat liver. J Biol Chem 266:12502–12507.

Van Veldhoven PP, Brees C, Mannaerts GP. 1991. D-Aspartate oxidase, a peroxisomal enzyme in liver of rat and man. Biochim Biophys Acta 1073:203–208.