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TWO PROTEIN FRACTIONS OBTAINED FROM HEPATIC PLASMA MEMBRANES

STUDIES OF THEIR COMPOSITION AND DIFFERENTIAL TURNOVER

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SUMMARY

1. Plasma membranes were isolated from rat liver and their enzymatic, morphologic, and chemical composition was studied. Two protein fractions were isolated from a hepatic plasma membrane preparation using either 33 % aqueous pyridine or 0.05 M K_2CO_3. One was water-soluble, contained 50 % of the original membrane protein; the other was water-insoluble, contained the remaining protein and the majority of lipids. With 0.05 M K_2CO_3 the majority of sialic acid was in the water-insoluble fraction. Neutral carbohydrates were equally distributed between the two fractions.

2. Polyacrylamide gel electrophoresis of water-soluble fractions in sodium dodecyl sulfate revealed 15–20 protein bands which were also present in the intact plasma membrane.

3. Relative protein turnover was determined by a double isotope technique. The water-insoluble fraction had a greater turnover ratio than the water-insoluble fraction. These studies are consistent with a "multistep process" of plasma membrane synthesis.

INTRODUCTION

Studies of hepatic endoplasmic reticulum membranes indicate that its pulse-labeled proteins undergo differential degradation and suggest that endoplasmic membrane synthesis is a multistep process[1,2]. Comparable observations have not been made regarding the synthesis and assembly of hepatic cell plasma membranes, although RAY *et al.*[3] demonstrated that proteins may be formed several hours before they appear in liver plasma membrane. In order to determine whether protein components of the liver plasma membrane are synthesized simultaneously ("one-step assembly"), or if incorporation and degradation of labeled proteins are heterogeneous("multi-step assembly"), we have prepared two protein fractions from liver plasma membrane preparations using aqueous pyridine and K_2CO_3. Half of the plasma membrane protein was water-soluble and lipid-free with a turnover ratio smaller than that observed for the remaining protein fraction, which was water-insoluble and contained the majority of

the lipid. These studies are compatible with a multistep assembly process for protein components of liver plasma membrane.

MATERIALS AND METHODS

All chemicals were reagent grade unless otherwise specified. Pyridine was purified by distillation.

Preparation of plasma membranes

Hepatic plasma membranes were prepared by the method of NEVILLE[4] except for addition of liver perfusion and washing of the membrane preparation. Male Sprague–Dawley rats, weighing 250–350 g were fed Purina rat chow *ad libitum*. Approx. 30 g of liver was removed from three rats under ether anesthesia and perfused through the hepatic veins with ice-cold 0.25 M sucrose containing 1 mM $MgCl_2$ and 1 mM $NaHCO_3$. Liver perfusion did not reduce plasma membrane purity or yield. The final preparation was washed 3 times in distilled water to remove excess sucrose, $NaHCO_3$, and loosely bound proteins. The purity of each membrane preparation was followed during the procedure by phase contrast microscopy and preparations containing more than one nucleus per low-power field were discarded.

Rat erythrocyte membranes were prepared from freshly drawn blood as previously described by DODGE *et al.*[5], and dialyzed for 24 h against distilled water to remove excess sodium phosphate buffer[6]. These rat erythrocyte ghosts were treated with 33 % aqueous pyridine as described by BLUMENFELD *et al.*[6] and the intact membranes, water-soluble and water-insoluble fractions compared with similarly prepared fractions from hepatic cell plasma membranes using dodecyl sulfate acrylamide gel electrophoresis.

Electron microscopic observation

Pellets of liver plasma membrane preparations were fixed in 3 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) overnight at 0–4°, (ref. 7) post-fixed in 1 % osmic acid in Millonig's 0.1 M phosphate buffer (pH 7.2)[8] and embedded in Epon[9]. Lead-stained[10] thin sections were examined in a Siemens Elmiskop I electron microscope.

Enzyme assays

5′-Nucleotidase (EC 3.1.3.5) using both AMP and CMP as substrate[11], IDPase (EC 3.6.1.6)[11], Mg^{2+}–ATPase (EC 3.6.1.3)[11], alkaline phosphatase (EC 3.1.3.1)[12] using β-glycerolphosphate as substrate and glucose-6-phosphatase (EC 3.1.3.9)[13] activities were measured in liver homogenate and plasma membrane preparations. Enzyme activities were determined by the initial rate of release of phosphate[14] from appropriate substrates at 37°, and expressed as μmoles of phosphate released per mg protein per h.

Chemical determinations

Protein was determined by the method of LOWRY *et al.*[15] using bovine serum albumin as standard, and by the micro-Kjeldahl nitrogen determination assuming an average nitrogen content of 16 %. Amino acid analysis was performed using a Beckman Model 120B amino acid analyzer following hydrolysis of protein in 6 M HCl for 22 h at 105° *in vacuo*. Organic phosphorus was measured by the method of BARTLETT[16]

and cholesterol, following saponification[17], by the method of ZLATKIS *et al.*[18] using crystalline cholesterol as the standard. Neutral sugars were assayed by the anthrone reaction[19] using glucose as the standard and *N*-acetylneuraminic acid by the thiobarbituric acid method of WARREN[20].

Solubilization of plasma membranes

One-half volume of ice-cold pyridine was added to one volume of washed liver plasma membranes suspended in distilled water at approx. 2.5 mg protein per ml[21]. The clear solution was immediately dialyzed against a 25-fold volume of distilled water for 15 h at 4°. Prolonged or exhaustive dialysis was avoided as this led to precipitation of all proteins. The turbid solution was centrifuged in a Spinco Model L centrifuge at 100000 × *g* for 60 min. The clear supernate (pyridine–water-soluble fraction) was dialyzed against a 25-fold volume of distilled water at 4° for 4 h to remove residual pyridine. The pellet was washed 3 times with 7 ml of distilled water (pyridine–water-insoluble fraction).

Washed hepatic plasma membrane preparations were also treated with 0.05 M K_2CO_3 (pH 11.2) as described by NEVILLE[22]. One-tenth volume of 0.5 M K_2CO_3 was added to one volume of washed plasma membranes suspended in distilled water at approx. 2.5 mg protein per ml, with gentle shaking for 30 min at room temperature. The turbid solution was centrifuged in the Spinco Model L centrifuge at 100000 × *g* for 60 min, yielding a clear supernate (K_2CO_3–water-soluble fraction) and a pellet (K_2CO_3 –water-insoluble fraction) which was washed 3 times with 7 ml of 1 mM $NaHCO_3$.

Polyacrylamide gel electrophoresis

5 % acrylamide gels containing 0.1 % sodium dodecyl sulfate were prepared and utilized as described by MAIZEL[23]. Gel columns were 100 mm in length with an internal diameter of 5 mm. All samples were pretreated prior to electrophoresis by dialysis against 0.01 M phosphate buffer (pH 6.8) containing 1 % sodium dodecyl sulfate and 1 % β-mercaptoethanol for at least 24 h at room temperature. An aliquot containing 100–150 μg protein in less than 0.3 ml was heated at 100° for 1 min. Electrophoresis was performed at room temperature in 0.1 M phosphate buffer (pH 6.8) containing 0.1 % sodium dodecyl sulfate for 3.5 h at 10 mA per gel (approx. 6 V/cm). The gels were fixed in 10 % trichloroacetic acid for 16 h, stained with 0.25 % Coomasie Blue for 2 h, and destained in 7 % acetic acid. Immunoglobulin G (mol. wt. 160000), albumin (mol. wt. 67000), ovalbumin (mol. wt. 45000), chymotrypsinogen A (mol. wt. 25000) and myoglobin (mol. wt. 17800) (obtained from Mann Research Lab.) were each subjected to electrophoresis in the presence of bromphenol blue. A plot of the logarithm of molecular weight *vs.* relative migration was calculated on semi-logarithm paper[24] and molecular weights were determined from this curve. In several studies gels were stained in parallel for glycoproteins and glycolipids using a periodic acid–Schiff stain as described by ZACHARIAS *et al.*[25]. These gels were destained with 7 % acetic acid instead of water.

Isotope studies

The turnover ratio $^3H/^{14}C$ was determined in the proteins derived from liver homogenate, intact plasma membrane, water-soluble and water-insoluble membrane fractions as described by ARIAS *et al.*[1]. Uniformly labeled leucine, L-[$^{14}C_6$]leucine (specific activity 278 mC/mmole), and L-[4,5-$^3H_2$]leucine (specific activity 44 C/mmole)

(obtained from New England Nuclear Corporation) were diluted with normal saline to 25 μC/ml and 100 μC/ml, respectively, and administered intraperitoneally in volumes of 1 ml or less. 20 μC of L-[$^{14}C_6$]leucine was administered to normal male Sprague–Dawley rats weighing 250–300 g followed 36 h later by 100 μC of L-[4,5-$^{3}H_2$]leucine and animals were killed 12 h later. The liver from a labeled animal was combined with the livers of two unlabeled pair-fed animals to give a final total liver weight of 30 g. Control studies were carried out with the same protocol except that 80 μC of [4,5-$^{3}H_2$]-leucine and 20 μC of L-[$^{14}C_6$]leucine were injected simultaneously 12 h prior to sacrifice. Hepatic plasma membranes were prepared and solubilized as previously described. All double-isotope studies were performed in duplicate in two groups of three rats each.

Samples containing 1–2 mg protein were precipitated with an equal volume of ice-cold 10 % trichloroacetic acid and collected on glass fiber disks (Whatman). Samples were serially washed with 5 ml each of ethanol, ether, and acetone; and finally with 15 ml of ice-cold 5 % trichloroacetic acid. The disks were dried by negative pressure and transferred to counting vials to which 0.5 ml of Soluene (Packard Inst. Co.) was added. They were allowed to digest at room temperature for 12 h. 10 ml of a toluene–Omnifluor (New England Nuclear Corp., containing 98 % 2,5-diphenyloxazole and 2 % Bis-MSB, 4 g/l toluene) solution were added to the dissolved precipitates and radioactivity in the resulting mixture was determined in a Nuclear-Chicago 720 refrigerated scintillator spectrometer. The efficiency of counting was 24 % for ^{3}H and 50 % for ^{14}C. Insignificant ^{3}H activity was present in the ^{14}C channel. Counting was performed to at least 5 % accuracy and quenching was determined by internal standardization. Quenching was minimal and did not differ from sample to sample. The turnover ratio was expressed as the $^{3}H/^{14}C$ ratio in each protein fraction. In order to correct for differences in body weight, amount of isotope administered and free amino acid pools, a turnover index was determined by dividing the $^{3}H/^{14}C$ ratio of each fraction[26].

RESULTS

Hepatic plasma membrane isolation

Purity of intact hepatic plasma membrane preparations was evaluated by phase and electron microscopy, and biochemical markers. On phase microscopy, the plasma membrane preparations appeared as previously described by NEVILLE[27] and contained less than one nucleus per every fifth low-power field. Electron micrographs revealed sheets of membranes connected by junctional complexes, and various sizes of membrane-enclosed vesicles (Fig. 1). Rarely, fragmented microbodies or lysosomes were seen.

In the plasma membrane fraction there was an enrichment in specific activity of enzymes thought to be associated with this fraction (Table I). With AMP and CMP as substrate for 5'-nucleotidase, a 20–26-fold increase in specific activities relative to the liver homogenate was found. The relative increase in specific activity of Mg^{2+}–ATPase (12-fold) and IDPase (10-fold) was less than that observed with 5'-nucleotidase possible because of the more diffuse distribution of these enzymes[20,29]. Alkaline phosphatase activity, not found by EMMELOT *et al.*[30] in the normal intact plasma membrane was present at levels reported by SONG *et al.*[31] in a plasma membrane fraction. Glucose-6-phosphatase activity was not detected in the plasma membrane preparation although the method used could detect 0.005 μmole of phosphate liberated per h per mg of pro-

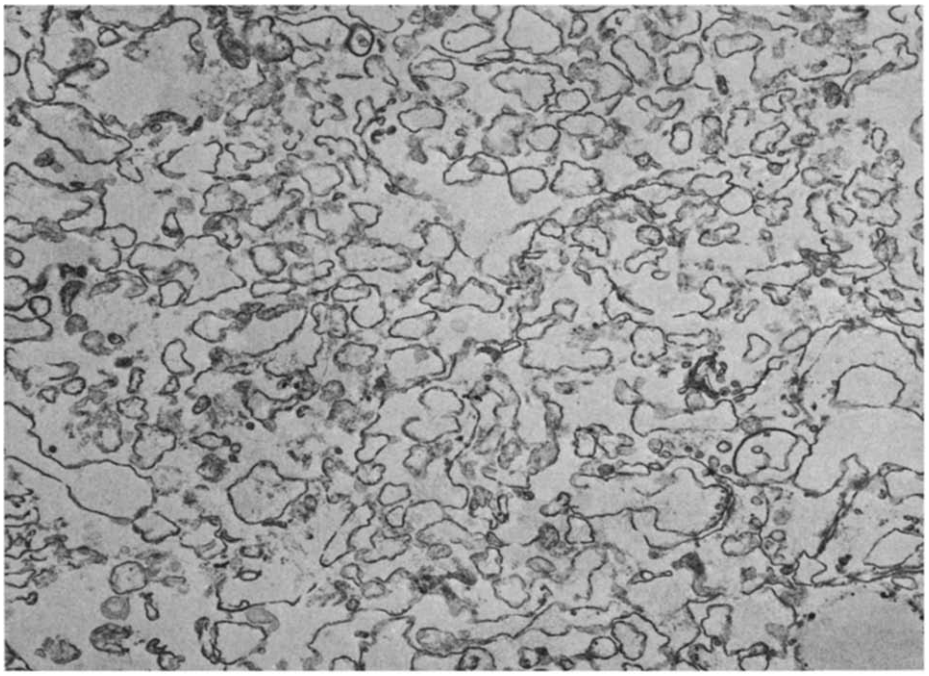

Fig. 1. Low-power (magnification 3870 ×) electron micrograph of isolated liver plasma membrane preparation demonstrating sheets of membranes frequently connected by tight junctions and various sizes of membrane-enclosed vesicles. Section stained with lead.

TABLE I

ENZYMATIC ACTIVITIES IN RAT LIVER PLASMA MEMBRANE

The activity of enzymes was measured by the release of phosphate at 37° during the time of zero-order kinetics using appropriate substrates. Specific activity is defined as μmoles of phosphorus released per mg protein per h. The increase in specific activity of enzyme activity is determined by the membrane/homogenate specific activities ratio. The figures in parentheses indicate the number of experiments done on different preparations. n.d. = no detectable activity. Enzyme assays were run in duplicate with appropriate tissue and substrate blanks.

Enzymes	*Specific activity (μmoles/mg protein per h)*		*Increase in specific activity (membrane/homogenate)*
	Homogenate	*Membrane*	
5′-Nucleotidase			
AMP (4)	1.8	46.4	26
CMP (4)	1.4	26.2	19
Mg^{2+}-ATPase (4)	2.6	30.0	12
IDPase (4)	3.7	36.8	10
Alkaline β-glycerophosphatase (2)	0.2	0.8	4
Glucose-6-phosphatase (6)	2.7	n.d	—

tein with glucose 6-phosphate as substrate. Following pyridine or K_2CO_3 treatments, enzyme activities were not detected most likely due to enzyme inactivation.

As outlined above, two modifications were introduced to the preparation of

hepatic plasma membranes originally described by NEVILLE[4]. Perfusion of rat liver removed most of the red cells and serum proteins and consistently gave white preparations instead of the salmon-pink preparations obtained without liver perfusion. Secondly, the final plasma membrane preparation was washed 3 times with distilled water to remove loosely bound proteins. Deionized distilled water removed these proteins as effectively as was reported by EMMELOT *et al.*[30] using normal saline. The use of distilled water led to greater yields of water-soluble proteins in the pyridine and K_2CO_3 solubilization procedures. After liver perfusion, only 5 % of membrane protein was removed in distilled water as compared to 20–25 % without liver perfusion. This is consistent with previous studies showing the presence of hemoglobin[32], serum proteins[30] and cytoplasmic proteins[33] bound to membranes obtained from livers which were not perfused.

Plasma membrane solubilization and chemical characterization of two protein fractions

The intact liver plasma membrane was treated with 33 % aqueous pyridine or 0.05 M K_2CO_3. The addition of either resulted in two distinct fractions. One was water-soluble and contained 50 % of the membrane proteins and a small percentage of total cholesterol and phospholipid. The other was water-insoluble and contained the remaining protein and the majority of the lipids (Table II). Neutral sugars were equally distributed between the two fractions. Sialic acid distribution was different with the two methods of solubilization; aqueous pyridine resulted in equal distribution between the two fractions, while the majority of sialic acid was found in the water-insoluble fraction after K_2CO_3 treatment.

TABLE II

CHEMICAL COMPOSITION OF LIVER PLASMA MEMBRANE PREPARATION AND DISTRIBUTION OF COMPONENTS

Liver cell plasma membranes prepared by the method of NEVILLE[4] were washed with distilled water and the chemical composition and distribution determined in duplicate for the plasma membrane and its fractions. The numbers in parentheses represent numbers of experiments done on different preparations.

Composition	*Intact plasma membrane (6)*		*Percent distribution*			
	% dry wt.	*μg/mg protein*	*Water-soluble fraction*		*Water-insoluble fraction*	
			Pyridine (3)	*K_2CO_3 (2)*	*Pyridine (3)*	*K_2CO_3 (2)*
Protein	51	—	49	51	31	37
Cholesterol	11.5	237	6	1	80	90
Phosphorus	0.6	12.9	11	9	70	75
Neutral sugars	4.3	95	30	34	39	52
Sialic acid	0.7	13.5	25	8	36	61

This chemical distribution of membrane components is similar to that observed previously in the human erythrocyte membrane, except that sialic acid was not selectively found in the water-soluble protein fraction. The amino acid composition of the two pyridine fractions (Table III) showed no important differences and generally appeared similar to that found by ourselves and others in the intact plasma membrane[34,35].

TABLE III

AMINO ACID COMPOSITION OF INTACT LIVER PLASMA MEMBRANE AND AQUEOUS PYRIDINE FRACTIONS

Amino acid analysis performed on two preparations of washed liver plasma membranes and its two fractions following aqueous pyridine treatment. Each sample was hydrolyzed for 22 h at 105° in 6 M HCl and recorded as the average of the two samples. Tryptophan, cysteine, half-cystine and methionine were not measured.

Amino acid	*Residues per 1000 residues*		
	Intact membrane preparation	*Water-soluble fraction*	*Water-insoluble fraction*
Lysine	68	74	67
Histidine	22	24	17
Arginine	50	48	52
Aspartic	78	104	94
Threonine	62	55	56
Serine	82	81	97
Glutamic	113	118	102
Proline	75	50	64
Glycine	88	78	90
Alanine	86	83	79
Valine	62	64	64
Isoleucine	48	47	51
Leucine	98	103	104
Tyrosine	25	30	20
Phenylalanine	43	41	42

The low protein recoveries of components seen with both methods of solubilization are probably related to losses of insoluble pellet material during washing procedures[23]. The relatively low recovery (62–69 %) of sialic acid in both procedures cannot be explained at the present time.

Polyacrylamide gel electrophoresis

In order to separate and further characterize the membrane proteins, intact plasma membrane preparations and water-soluble and water-insoluble fractions were subjected to electrophoresis in polyacrylamide gels which contained 0.1 % sodium dodecyl sulfate. In the presence of sodium dodecyl sulfate, separation is a function of molecular weight[25,35]. Fig. 2 shows protein gel patterns obtained with intact plasma membrane preparation and the two protein fractions obtained with pyridine and K_2CO_3. At least 15–20 major bands were seen in the soluble fractions, the majority of which correspond to bands seen in the intact plasma membrane preparation. The insoluble protein fractions showed less penetration into the sodium dodecyl sulfate polyacrylamide gels, however, similarities in gel patterns were seen when compared to the intact membrane preparations and soluble fractions. Protein bands unique to each fraction were present. Staining of duplicate gels with periodic acid–Schiff stain resulted in the staining of three bands in the intact plasma membrane. The slower two bands correspond to proteins of mol. wt. 120000 and 45000. Their interrelationship is unknown. The faster moving of the three periodic acid–Schiff staining bands does not correspond to a Coomasie Blue-staining protein band and probably represent glycolipids as described by LENARD[37]. The fast moving band was observed in both water-insoluble fractions,

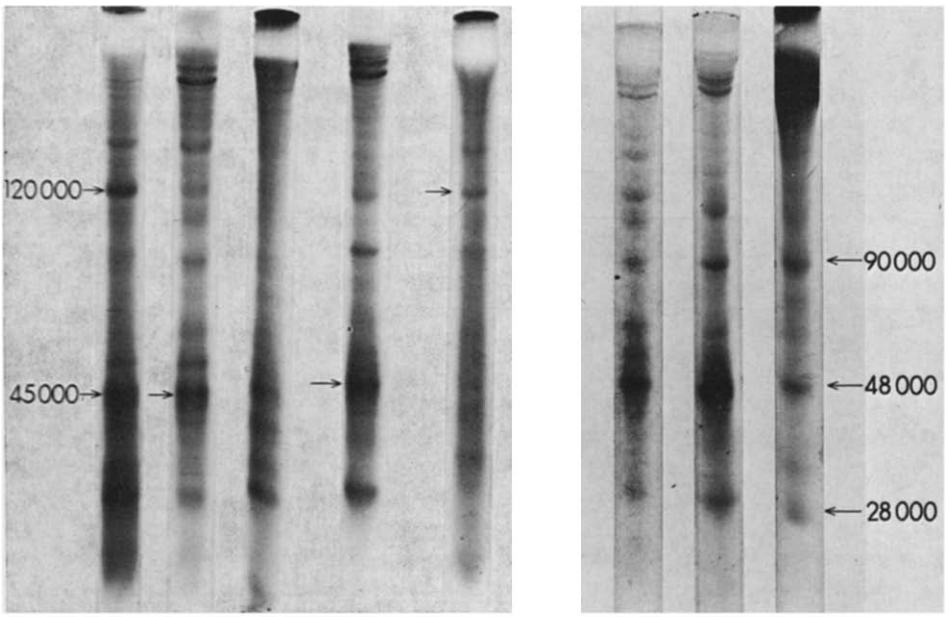

Fig. 2. Polyacrylamide gel electrophoresis in 0.1 % sodium dodecyl sulfate. Protein samples of 100–150 μg treated with 1 % sodium dodecyl sulfate and 1 % β-mercaptoethanol in 0.01 M phosphate buffer and heated to 100° for 1 min were applied to 5 % acrylamide – 0.1 % sodium dodecyl sulfate gel 10 cm in length and run at 10 mA per gel from anode (top) to cathode (bottom) for 3.5 h. After fixing in 10 % trichloroacetic acid, gels were stained with Coomasie Blue. Samples from left to right are intact plasma membrane, pyridine–water-soluble, pyridine–water-insoluble, K_2CO_3–water-soluble and K_2CO_3–water-insoluble. Arrows point to corresponding periodic acid–Schiff staining bands which correspond to protein bands. Molecular weights were estimated from the log plots of molecular weights of known proteins *vs.* their relative migration.

Fig. 3. Samples and polyacrylamide gel electrophoresis run as described in Fig. 2. Samples from left to right are liver pyridine–water-soluble, liver K_2CO_3–water-soluble and rat erythrocyte pyridine–water-soluble. Arrows point to similar band mobilities in the erythrocyte and liver water-soluble fractions. Molecular weights were estimated as described under Fig. 2.

but neither of the two water-soluble fractions. In both water-soluble fractions only one band was stained corresponding to a protein of mol. wt. 45000. A different pattern was observed in the K_2CO_3–water-insoluble fraction, which demonstrated a periodic acid–Schiff staining band mol. wt. 120000. Fig. 3 illustrates the protein pattern obtained on sodium dodecyl sulfate acrylamide gel electrophoresis of water-soluble proteins from rat erythrocyte membranes. In comparison with the water-soluble protein fractions of hepatic plasma membrane, many bands with different mobility were present. However, at least three protein bands with mol. wt. 28000, 48000 and 90000 were seen in both the liver and erythrocyte water-soluble fractions. With the exception of these three protein bands, the membrane proteins derived from rat liver and erythrocyte appear to be unique to each fraction. These proteins have not been further characterized.

The similarities of protein patterns observed in the water-soluble proteins obtained with pyridine or K_2CO_3 to those observed with intact membrane preparations suggest that these methods of treatment do not lead to major alterations in membrane

proteins. The procedures appear to result in similar protein separations although certain protein bands are unique to each method of solubilization.

Radioisotope incorporation studies

The determination of the first-order rate of degradation of a protein normally involves administration of a single dose of labeled amino acid and subsequent determination of the loss of isotope in the protein at a number of time points. In contrast, the procedures employed here require determination of only two points along a decay curve. The two points were identified by the use of different isotopes of the same amino acid. This technique does not allow for an estimate of absolute rates of degradation, but permits one to establish relative rates of protein turnover. As employed here, L-[$^{14}C_6$]leucine was administered at zero time and pulse-labeled proteins were allowed to decay 2 days, thus establishing a 48-h time point. L-[4,5-$^{3}H_2$]Leucine was administered to the same rat and the animal was sacrificed 12 h later. Thus ^{3}H radioactivity represents a 12-h or initial time point on a standard decay curve. Therefore, proteins with relatively rapid turnover rates have relatively high $^{3}H/^{14}C$ ratios.

In the performance of these studies four assumptions were made[1]: (1) At the time the animal is killed the labeled proteins are in a process of isotopic decay. Others[3] have reported that peak incorporation with rat liver plasma membrane protein was reached at 4 h and by 8 h the labeled proteins were in isotopic decay. In our studies, animals were killed 12 h after the second injection of isotope in order to be certain that labeled proteins are in isotopic decay. If this requirement is not met, the $^{3}H/^{14}C$ ratios would not reflect degradation rates but a combination of degradation and the time required for passage of the proteins into the membrane. (2) Labeled leucine is not metabolized to other amino acids. Following hydrolysis of the intact liver plasma membrane preparation in 6 M HCl, *in vacuo* for 22 h at 105°, an aliquot was chromatographed on silica gel thin-layer chromatography sheets (obtained from Brinkmann Instruments, Inc.). The amino acid hydrolysate was chromatographed in two dimensions; first for 90 min in chloroform–methanol–17 % ammonia (2:2:1, by vol.) and, in a second solvent system of phenol–water (75:25, by vol.) for 240 min[38]. 0.5 cm × 0.5 cm cut sections were determined for radioactivity by scintillation spectrometry as previously described. Radioactivity was confined only to the leucine–isoleucine region. In addition the trichloroacetic acid precipitates were washed with ethanol, acetone, ether, and 5 % trichloroacetic acid to remove lipids, nucleic acids and carbohydrates. (3) Labeled proteins of the hepatic plasma membrane follow first-order decay kinetics. Most proteins studied have been found to follow exponential decay kinetics. The only known exception is hemoglobin which follows "life-span" kinetics. That the labeled proteins of the intact liver plasma membrane undergo exponential decay has been demonstrated[1,39]. (4) The rates of synthesis of plasma membrane proteins are similar at the time of both injections of labeled leucine. Nutritional status, which has been shown to alter turnover of proteins[40] was unchanged during the study.

The turnover ratio and index in rat liver homogenate, intact plasma membrane, and membrane fractions from control and experimental animals is shown in Table IV. There was little variation in the $^{3}H/^{14}C$ ratios in the control samples which were given [^{3}H]leucine and [^{14}C]leucine simultaneously. In the turnover studies, labeled proteins of the intact plasma membrane had a higher turnover ratio and index compared to liver homogenate protein. Previous studies have also shown that the half-life of plasma

TABLE IV

RELATIVE TURNOVER OF PLASMA MEMBRANE PROTEINS

Sprague–Dawley rats weighing 250–300 g were given 20 μC L-[$^{14}C_6$]leucine initially. 36 h (Expts. A and B) later, each rat was given 100 μC L-[4,5-$^{3}H_2$]leucine and the animals were killed 12 h later. In control experiments 20 μC L-[$^{14}C_6$]leucine were administered simultaneously 12 h prior to sacrifice. In all studies unlabeled rat liver was combined with the labeled liver to total 30 g and plasma membranes were prepared by the method of NEVILLE[4] and water-soluble and water-insoluble fractions isolated as described in text. Relative turnover rates are expressed as $^{3}H/^{14}C$ counts ratios and the turnover index as the ratios of plasma membrane and its fractions divided by the homogenate ratio. A high turnover ratio or index indicates a more rapid rate of turnover. Each value is of duplicate samples counted to 5% efficiency.

Cell fraction	$^{3}H/^{14}C$ *ratio*			*Index (cell fraction ratio)/(homogenate ratio)*			
		Expt.			*Expt.*		*Av. index (Expts. A + B)*
	Control	*A*	*B*	*Control*	*A*	*B*	
Homogenate	3.7	9.7	7.1	1.00	1.00	1.00	1.00
Intact plasma membrane	3.5	11.0	8.5	0.95	1.14	1.20	1.17
Water-soluble fraction							
Pyridine	3.7	10.1	7.5	1.00	1.04	1.06	1.05
K_2CO_3	—	8.9	7.9	—	0.92	1.11	1.02
Water-insoluble fraction							
Pyridine	3.6	12.1	8.6	0.97	1.25	1.21	1.23
K_2CO_3	—	12.8	9.3	—	1.32	1.31	1.32

membrane protein (3.1 days) was shorter than that observed for the homogenate protein (4.5 days)[2]. The plasma membrane fractions prepared by either aqueous pyridine or K_2CO_3 were observed to have different turnover ratios. The water-insoluble protein fraction had an average turnover ratio greater than that found in the intact membrane, and both were greater than that found in the water-soluble membrane fraction. This differential turnover is consistent with the separation of two groups of proteins by different methods of protein solubilization.

DISCUSSION

Studies of the synthesis, degradation, and assembly of constituents of the liver cell plasma membrane present difficulties because of limited recovery of membrane and inability to separate or isolate its protein components. Although pulse-labeled triglycerides of rat liver plasma membrane appear to have a faster turnover than pulse-labeled proteins[39], heterogeneity of membrane protein components has not been demonstrated.

The plasma membrane preparation used in this study had enhanced specific activity of 5'-nucleotidase and alkaline phosphatase, but no glucose-6-phosphatase activity suggesting that the membranous structures observed on electron microscopy are predominantly of plasma membrane origin with little microsomal contamination. Perfusion of the liver and washing of membranes with distilled water removed most serum, basic cytoplasmic proteins and hemoglobin. Chemical analysis of this preparation was similar to those previously reported[30, 32, 35, 42].

Solubilization of membrane proteins generally involves the use of organic solvents[43–45], detergents[46–48] or concentrations of salt[49]. The presence in human erythrocyte membrane of two classes of proteins differing in water solubility and affinity for lipid has been shown, using several methods of solubilization[6,47]. In this study two such classes of proteins are also present in the liver plasma membrane. Using aqueous pyridine and K_2CO_3 solubilization, 50 % of the liver plasma membrane protein becomes water-soluble and lipid-free. However, the sialic acid-containing components of the liver plasma membrane differed in their distribution suggesting that these sialoproteins have different properties than those of the erythrocyte membrane.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis revealed that intact plasma membrane and the two water-soluble fractions were separated into 15–20 protein bands with a wide range of molecular weights, similar to results described for the erythrocyte[6,37,50], mitochondrial, and endoplasmic membranes[51,52]. Many similarities are seen in protein and periodic acid–Schiff staining patterns in the two water-soluble fractions. Whether or not the protein band patterns of the water-insoluble fractions are similar is difficult to conclude because a large portion of the protein did not enter the gel.

Degradation of protein fractions was determined by a double isotope ratio method[1] which permits estimation of relative degradation rates. These studies demonstrate that the liver plasma membrane contains two groups of proteins, one with a relatively fast turnover rate and the other with a relatively slower rate. The observation that different protein turnover ratios are found for two plasma membrane fractions is consistent with a "multi-step assembly" model of plasma membrane synthesis as has previously been proposed for the endoplasmic reticulum[2]. Similarities in turnover ratios, as well as chemical characterization and electrophoretic band patterns of water-soluble proteins solubilized by two different methods, suggest that proteins having common properties are present in this fraction.

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