Biosynthesis of Liver Membranes

INCORPORATION OF [^3H]LEUCINE INTO PROTEINS AND OF [^{14}C]GLUCOSAMINE INTO PROTEINS AND LIPIDS OF LIVER MICROSOMAL AND PLASMA-MEMBRANE FRACTIONS

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1. The smooth- and rough-microsomal and the light and heavy plasma-membrane fractions of mouse liver homogenates were prepared and characterized by using biochemical markers. 2. The hexosamine/protein ratio was threefold higher in the plasma membranes than in the smooth-microsomal fraction. Glucosamine was bound only to protein, and galactosamine was attached mainly to lipids. 3. [^3H]-Leucine and [^{14}C]glucosamine were injected into animals and the rates of incorporation of radioactivity into the fractions were determined. Both precursors were rapidly incorporated into the microsomal fractions, but plasma membranes showed a slower rate of synthesis which reached a maximum at 2-4 h after intravenous administration. 4. The light- and heavy-plasma-membrane fractions showed similar patterns of incorporation, and therefore a precursor-product relationship appears unlikely. 5. Plasma membranes, especially the light subfraction, showed appreciable incorporation of hexosamine into chloroform-methanol-soluble components which were shown to be mainly glycolipids. 6. The results indicate that liver plasma-membrane proteins and glycoproteins are synthesized at similar rates. However, glycolipid synthesis in plasma membranes occurred more rapidly.

The plasma (surface)-membrane fraction prepared from rat or mouse liver homogenates is heterogeneous and two or more plasma-membrane subfractions which possess characteristic enzymic, chemical and morphological properties have been described (Evans, 1969, 1970a; House & Weidemann, 1970; Bock, Siekevitz & Palade, 1971). Whereas the major plasma-membrane subfraction of density 1.18 (heavy subfraction) possesses sheet-like membrane fragments that have intercellular junctions, e.g. tight junctions and desmosomes, the light subfraction (density 1.13) consists of smooth vesicles only, and shows the highest enrichment of 5'-nucleotidase as well as other marker enzymes, histocompatibility antigens and cholesterol and sialic acids (Evans, 1970a; Evans & Bruning, 1970). Electrophoresis in polyacrylamide gels has shown that there are only minor differences in the protein composition of light and heavy subfractions, in contrast with the major differences apparent between the proteins and glycoproteins of plasma and smooth endoplasmic reticulum membranes (Evans, 1970b). Because of the gross chemical similarity of the two plasma-membrane subfractions, it is possible that the light fraction is a biosynthetic precursor of the heavy fraction. To

examine this possibility, and also to investigate in general the biosynthetic relationship between liver plasma and intracytoplasmic membranes, we have measured the incorporation of intravenously administered precursors of protein and carbohydrate into microsomal and plasma-membrane fractions isolated from the same tissue homogenate.

The results reported in the present paper show that there are temporal differences in the incorporation of [^3H]leucine and [^{14}C]glucosamine into the protein and carbohydrate components of the intracytoplasmic and the plasma membranes. The light and heavy plasma-membrane fractions had similar rates of uptake of both precursors, thus supporting the idea that the light fraction originates from the cell surface. Appreciable incorporation of hexosamine into the glycolipids of the plasma membranes was found, thus emphasizing the active metabolic state of the cell-surface glycolipids.

EXPERIMENTAL

Administration of radioactive isotopes. For each time-point 35 Parkes' White mice (25g) that had been fed were used to prepare the subcellular fractions. Ten mice were injected via the tail vein with 0.4 ml of a solution

Scheme 1. Preparation of microsomal and plasma membrane fractions

containing $300\mu\text{Ci}$ of $[^3\text{H}]\text{leucine}$ and $60\mu\text{Ci}$ of $[^{14}\text{C}]\text{-glucosamine}$ dissolved in 4.4 ml of phosphate-buffered saline. In one experiment in which the membrane fractions were examined by polyacrylamide-gel electrophoresis $600\mu\text{Ci}$ of $[^3\text{H}]\text{leucine}$ and $100\mu\text{Ci}$ of $[^{14}\text{C}]\text{-glucosamine}$ were administered. Mice were killed at the intervals specified (30 min–6 h) after injection of isotopes and then livers (total wt./experiment 50–65 g) from labelled and unlabelled animals were pooled for preparing the tissue homogenate.

Preparation of subcellular fractions. The fractions were prepared as shown in Scheme 1. The plasma-membrane preparative procedure with the MSE A XII zonal rotor and the subfractionation technique have been described (Evans, 1970a, 1971). Mitochondrial and microsomal fractions were prepared from the particulate material remaining in the $1000g$ supernatant by centrifuging under iso-osmotic conditions. The microsomal fraction was separated into rough and smooth components by the method of Dallner, Siekevitz & Palade (1966) by using the Beckman SW 27 rotor.

Lipid and carbohydrate analysis. Portions of the smooth-microsomal fraction and light and heavy plasma-membrane fractions were washed once with iso-osmotic sucrose and the wet pellets were dispersed in 4 ml of chloroform-methanol (2:1, v/v) and left overnight at room temperature. After low-speed centrifugation, the supernatants were removed and the pellet was re-dispersed in 2 ml of chloroform-methanol (2:1, v/v) and left for

30 min before centrifugation. The pooled extracts and pellets derived from approx. 5 mg of membrane protein were hydrolysed for 4 h in 4 M-HCl at 110°C *in vacuo*, and glucosamine and galactosamine were determined in an EEL amino acid analyser as described by Fanger & Smyth (1970). Chloroform-methanol extracts were partitioned further against an aqueous phase (Folch, Lees & Sloane-Stanley, 1957) and the radioactivity extracted into the organic and aqueous phases was determined as described below. The lipids in the organic and aqueous phases were qualitatively examined by t.l.c. on silica gel G plates (E. Merck A.G., Darmstadt, Germany) developed in the solvent system of Jatzkewitz & Mehl (1960) and stained with H_2SO_4 . Radioautograms of t.l.c. plates were prepared with Ilford No-Screen X-ray films. The incorporation of $[^{14}\text{C}]\text{-glucosamine}$ into amino sugars was determined after hydrolysis of membrane samples *in vacuo* for 4 h in 4 M-HCl at 110°C . The hydrolysate was dried, redissolved in 1 ml of m-HCl and added to a column ($5\text{ cm} \times 0.9\text{ cm}$) of Dowex 50 (X8) previously equilibrated with sodium formate. Columns were washed with 15 ml of water and amino sugars were eluted with 10 ml of 2 M-HCl (Boas, 1953). The acid eluates were dried and dissolved in a minimum volume of water and the amino sugars were separated by chromatography on Whatman no. 1 paper impregnated with 0.1 M-BaCl_2 (Heyworth, Perkins & Walker, 1961) by using butan-1-ol-pyridine-water (6:4:3, by vol.) as solvent. Amino sugars were detected with ninhydrin reagent and the spots correspond-

ing to glucosamine and galactosamine were cut out and their radioactivities measured by using the dioxan-based scintillant described below. Analysis of the smooth-microsomal and plasma-membrane fractions isolated at 1.5 and 4 h showed that hexosamines accounted for 80% of the incorporated radioactivity. Paper-chromatographic analyses of the plasma-membrane fractions indicated glucosamine/galactosamine radioactivity ratios of 2.5–3:1 and 4:1 at 1.5 and 4 h respectively.

Polyacrylamide-gel electrophoresis. Membrane fractions were dissolved in 5% (w/v) sodium dodecyl sulphate in 0.5 M-urea and boiled for 3–5 min. Electrophoresis was carried out at 4 mA/tube in 7% polyacrylamide gels containing 0.1% sodium dodecyl sulphate and 0.5 M-urea (Evans, 1970a). After electrophoresis, slices (1 mm long) of gel were dispersed in the apparatus of Maizel (1966) and radioactivity determined as described below.

Determination of radioactivity. Membrane fractions were washed once with iso-osmotic sucrose and then dissolved in 1 ml of M-NaOH after which 0.1 ml of conc. HCl was added. Scintillant consisting of a solution of 4 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene dissolved in 1 litre of xylene (10 ml) and 5 ml of Triton X-100 was added and radioactivity was measured in a Packard Tri-Carb scintillation counter. Counting efficiencies were: ^3H , 25%; ^{14}C , 70% and ^3H counts were corrected for channel spill-over. The radioactivities of dried samples of the organic extracts were counted in the same way. The radioactivity of polyacrylamide-gel fractions dispersed in 1 ml of water was counted in 10 ml of scintillant containing 180 g of naphthalene, 8 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene, dissolved in 1 litre of dioxan. Approx. 50% of the total radioactivity applied to the gels was recovered.

Other determinations. The protein content of membrane suspensions was determined by the method of Lowry, Rosebrough, Farr & Randall (1951). The RNA content of microsomal and plasma membranes was determined by the Schmidt-Thannhauser method described by Munroe & Fleck (1966). Glucose 6-phosphatase and 5'-nucleotidase activities were determined as described by

Evans (1970a). Glycosyltransferase activities were determined by measuring, in the presence or absence of Triton X-100 as indicated (Morré, Merlin & Keenan, 1969), the transfer of UDP-*N*-acetyl- ^{14}C glucosamine to endogenous acceptors or to the exogenous acceptor α_1 -acid glycoprotein that had been treated with sialidase, β -galactosidase and β -*N*-acetylglucosaminidase (Hughes & Jeanloz, 1964b). The acid glycoprotein was kindly provided by Dr R. C. Hughes of this Institute. Glucosidase and glucosaminidase activities of the plasma membrane fraction were determined as described by Hughes & Jeanloz (1964a).

Materials. All chemicals and reagents were of analytical grade and were obtained from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K., or BDH Chemicals Ltd., Poole, Dorset, U.K. L-[4,5- ^3H]Leucine (1.0 Ci/mmol) and D-[1- ^{14}C]glucosamine (0.3 Ci/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. UDP-*N*-acetyl[1- ^{14}C]glucosamine (10 μCi /0.142 mg) was obtained from New England Nuclear Corp., Boston, Mass., U.S.A. Glass-distilled water was used in all experiments.

RESULTS

Characteristics of subcellular fractions. The distribution of the marker enzymes glucose 6-phosphatase and 5'-nucleotidase and of RNA in the microsomal and plasma-membrane subfractions prepared according to Scheme 1 was determined (Table 1). Initial homogenization of liver in 1 mM-sodium hydrogen carbonate was necessary for the separation of the plasma membranes by the rate-zonal procedure. The results show an enrichment of 5'-nucleotidase in the plasma membrane fractions and glucose 6-phosphatase in the microsomal fractions. Whereas glucose 6-phosphatase appears to be exclusively located in the microsomal fraction, some 5'-nucleotidase activity was found in the smooth-microsomal fraction, in agreement with

Table 1. *Distribution of marker enzymes and RNA in mouse liver subcellular fractions prepared according to Scheme 1*

Fraction	5'-Nucleotidase (μmol of P_i hydro- lysed/h per mg of protein)	Glucose 6-phosphatase (μmol of P_i hydro- lysed/h per mg of protein)	Glycosyltransferase (d.p.m./h per mg of protein)		RNA (μg /mg of protein)
			Without added substrate	With added substrate	
Homogenate	0.6 (1)	1.4 (1)	450	40	N.D.
Microsomal	N.D.	4.3 (3.1)	N.D.	N.D.	N.D.
Smooth microsoma	2.7 (4.5)	6.2 (4.4)	1400	500	0.112
Rough microsomal	0.6 (1)	4.7 (3.4)	N.D.	400	0.312
Plasma membranes	14.0 (23)	0.6 (0.5)	N.D.	11	0.002
Light subfraction	25.2 (42)	N.D.	110	60	
Heavy subfraction	10.4 (17)	N.D.	160	0	

Glycosyltransferase determinations with and without added substrate were made with different preparations and with different amounts of UDP-*N*-acetyl[^{14}C]glucosamine. Reaction mixtures containing added substrate also contained 0.5% Triton X-100. N.D. = not determined. Values in parentheses indicate the extent of enrichment over homogenate.

Table 2. *Hexosamine content of liver smooth-microsomal and plasma-membrane fractions*

Fractions were extracted with chloroform-methanol (2:1, v/v) and the amino sugars determined on the organic phase and insoluble pellet as described in the Experimental section. Values are expressed as $\mu\text{mol/g}$ of protein.

Fraction	Soluble in chloroform-methanol		Pellet	
	Glucosamine	Galactosamine	Glucosamine	Galactosamine
Smooth microsomal	0	4.5	15.2	1.2
Heavy plasma membrane	0.5	21.3	35.2	2.7
Light plasma membrane	1.2	16.0	43.4	2.7

Bock *et al.* (1971). A threefold difference in the RNA content of the rough- and smooth-microsomal fractions was found.

The distribution of sugar transferases and hydrolases was also determined. The membrane fragments of the Golgi apparatus, which are now recognized as a major subcellular site of glycoprotein synthesis in liver, are found mainly in the smooth-microsomal fraction (Leelavathi, Estes, Feingold & Lombardi, 1970; Schachter *et al.* 1970). The results confirm the microsomal location of UDP-*N*-acetylglucosamine transferase activity; however, activity was also found in the rough-, as well as the smooth-microsomal fraction. Transferase activity measured in the presence or absence of exogenous acceptor could not be detected in the plasma-membrane fraction, in agreement with other reports (Wagner & Cynkin, 1969; Morré *et al.* 1969). Plasma-membrane fractions were also examined for glucosidase and glucosaminidase activities but no activity was detected. This is in contrast with the results of Fleischer & Fleischer (1969) who observed an enrichment of glucosidase activity in bovine plasma membranes.

Amino sugar content of membrane fractions. The amino sugar content of the lipid and protein components of the various membrane fractions was determined (Table 2). Both plasma-membrane fractions contain a threefold higher aminosugar content relative to unit protein weight than the smooth-microsomal fraction. Further, the results show the almost exclusive attachment of glucosamine to protein. In contrast, galactosamine is predominantly bound to lipid.

Incorporation of precursors into subcellular fractions. The incorporation of [^3H]leucine and [^{14}C]glucosamine into the nuclear pellet and supernatant is shown in Fig. 1. The zonal-rotor profiles of radioactivity and protein obtained for the nuclear pellet at 30 min and at 4 h are shown in Fig. 2. A higher incorporation into the fractions containing the plasma membranes is evident at the later time-period. At both times there is only a low degree of incorporation into fractions that contain predominantly nuclei and mitochondria.

Fig. 1. Time-course of incorporation of [^3H]leucine and [^{14}C]glucosamine into the 1000g pellet (O, Δ) and supernatant (●, ▲) respectively.

The rates of incorporation of both precursors into the smooth- and rough-microsomal fractions and unfractionated plasma membranes are shown in Fig. 3. Leucine incorporation occurs rapidly into both rough- and smooth-microsomal membranes, maximum values attained within 30 min of isotope administration being followed by a steady decline. In contrast plasma membranes do not attain maximum radioactivity until 1–1.5 h and this is then maintained for 3–4 h. Glucosamine incorporation into smooth-microsomal fraction reaches a sharp maximum at 90 min, whereas in plasma membranes a maximum is reached after 90 min, which then declines slowly in the following 4 h. Both plasma-membrane subfractions show similar patterns of leucine incorporation (Fig. 4). The [^3H]leucine and [^{14}C]glucosamine radioactivities of the homogenates that were recovered in the plasma-membrane fractions at three time-periods are shown in Table 3. If it is assumed that 5'-nucleotidase is mainly located in the plasma membranes of liver, it can be calculated on the basis of a 10% recovery of plasma membranes that 12–42% of the ^{14}C and 5–12% of the ^3H radioactivities were recovered in this fraction at 1.5–6 h respectively after isotope injection.

Fig. 2. Final rate-zonal centrifugation profiles of [^3H]leucine (●) and [^{14}C]glucosamine (○) from livers obtained at 30 min (a) and 4 h (b) after injection of isotopes. ▲, Protein content of fractions. Peak 1 contains soluble proteins and small membrane vesicles, peak 2 contains mitochondria, peak 3 contains plasma-membrane fragments and peak 4 contains nuclei and cell debris (Evans, 1970a).

The high specific radioactivity of ^{14}C incorporation observed in the plasma membranes relative to the smooth-microsomal fraction, prompted a closer investigation of the distribution of [^{14}C]hexosamine between the lipid and protein components of the plasma-membrane fractions. Whereas organic extracts of smooth-microsomal fractions never accounted for more than 12% of the total ^{14}C radioactivity, up to 50% of the counts was extracted from plasma membranes by chloroform-methanol (Fig. 4). Maximum incorporation into material extractable with the lipids of the light- and heavy-plasma-membrane fractions occurs between 90 and 180 min and is more marked in the light fraction. Therefore, although the incorporation pattern in the smooth-microsomal fraction is essentially unaltered by chloroform-methanol extraction, glycoprotein synthesis in plasma membranes is now seen to approach slowly a maximum at 3–4 h. The results therefore indicate that hexosamine is incorporated into two main classes of plasma-membrane components; glycolipids which reach a peak of incorporation at

90–180 min and glycoproteins which reach a maximum at 3–4 h.

Partitioning of the chloroform-methanol extracts of the three fractions against an aqueous phase (Folch, Lees & Sloane-Stanley, 1957) resulted in extraction of 50% of the radioactivity. Analysis of the organic and aqueous phases by t.l.c. and radioautography yielded similar patterns of labelling in the plasma membrane fractions (Plate 1). Labelled components are found exclusively near the origin where glycolipids migrate in this system (Payne, 1964). The results therefore confirm that the ^{14}C radioactivity extracted by chloroform-methanol is present as glycolipids.

Polyacrylamide-gel electrophoresis of membranes labelled for 4 h demonstrated the distribution of radioactivity in the protein and glycoprotein components (Fig. 5). The rough-microsomal membranes show incorporation of [^3H]leucine predominantly into low-molecular-weight components and a low degree of glucosamine incorporation. Smooth-microsomal, light- and heavy-plasma-membrane subfractions show appreciable

Fig. 3. Time-course of incorporation of [^3H]leucine (●) and [^{14}C]glucosamine (○) into (a) rough-microsomal fraction, (b) smooth-microsomal fraction and (c) unfractionated plasma membranes.

but differing amounts of incorporation of radioactivity from [^3H]leucine and [^{14}C]glucosamine. Low-molecular-weight peaks of high ^{14}C incorporation found at the gel front of the plasma-membrane fractions are indicative of glycolipid components (Lenard, 1970). These results confirm the presence in the plasma-membrane fraction of a number of glycoprotein components, in agreement with the observations made by Schiff-periodate staining of total membrane fractions (Evans, 1970b) and of pyridine extracts of plasma membranes (Gurd, Evans & Perkins, 1971) that were separated by gel electrophoresis.

DISCUSSION

The biosynthesis by rodent liver of exported and retained soluble proteins continues to be the subject of a large number of investigations (Redman, 1969; Hicks, Drysdale & Munroe, 1969; Glaumann & Ericsson, 1970; Campbell, 1970; Lo & Marsh, 1971). However, far less attention has been directed at the mechanism of synthesis of endoplasmic reticulum and plasma-membrane components. Before discussing the present results, it is pertinent to emphasize the compositional differences between the liver microsomal and plasma membrane fractions. Polyacrylamide-gel electrophoresis has shown microsomal and plasma membranes to be of widely different protein composition (Schnaitman, 1969; Evans, 1970b; Fleischer & Fleischer, 1970). In addition to the distribution of marker enzymes, entire multi-enzyme systems, e.g. those involved in microsomal reductive synthesis and detoxication, are absent from liver plasma membranes (Fleischer, Fleischer, Azzi & Chance, 1971). Plasma membranes are enriched in carbohydrates that are attached to either protein or lipid (Table 3). Further, there are noteworthy differences in the amounts of certain lipid components, especially cholesterol and sphingomyelin (Patton, 1970).

In the present paper we have measured the kinetics of incorporation of intravenously administered [^3H]leucine and [^{14}C]glucosamine into biochemically characterized microsomal and plasma-membrane fractions of mouse liver. The results show that maximum incorporation of [^3H]leucine into microsomal fractions occurs within 30 min of precursor administration whereas the plasma-membrane fractions show a slower rate of incorporation, reaching a maximum in 1.5 h which is maintained for 2–3 h. The pattern of labelling measured in the microsomal fractions in the first hour after injection of isotopes is a consequence of two or probably more concurrent processes. The rapid decrease of [^3H]leucine radioactivity in the smooth-microsomal fraction is a reflexion of the elaboration of proteins destined for export as well as the synthesis of new membranes. For example, Peters (1962) has shown that in rat liver, synthesis of serum albumin, which forms a major part of the protein-synthesizing activity, reaches a maximum in the rough- and smooth-microsomal fractions at 5 min and 20 min respectively, and most of the albumin is cleared from the liver in 50 min. Later time-points therefore give a truer indication of the rates of synthesis of the microsomal-membrane proteins and glycoproteins and allow comparisons to be made with the plasma-membrane fractions which bind only small amounts of soluble proteins (Evans, 1970a; Peters, 1962). These results, which

EXPLANATION OF PLATE I

T.l.c. of chloroform-methanol extracts (a) and their Folch washings (b) of smooth-microsomal fraction (SM), light-(PM-L) and heavy-(PM-H) plasma-membrane subfractions. Plates were developed and then stained with H₂SO₄ (A) after radioautography (B) as described in the Experimental section. Lipid markers indicated are CE, cholesterol ester, TG, triglyceride, C, cholesterol, PE, phosphatidylethanolamine, PC, phosphatidylcholine, SP, sphingomyelin. The fractions were isolated 90 min after the injection of isotopes.

Fig. 4. Time-course of incorporation of [³H]leucine (●) and [¹⁴C]glucosamine (○) into (a) light and (b) heavy plasma membranes. ○—○, ¹⁴C in chloroform-methanol-extracted membranes; ○-----○, ¹⁴C in chloroform-methanol extracts.

Table 3. Total radioactivities measured in liver homogenates and plasma-membrane fractions

In the preparation of plasma membranes, approx. 10–15% of the homogenate 5'-nucleotidase activity was recovered in the plasma-membrane fraction which contained 30–60 mg of membrane protein. Values in parentheses indicate the percentage of the homogenate radioactivity recovered in the individual fractions.

Time after injection of isotopes (h)	10 ⁻⁴ × Radioactivity (d.p.m.)							
	10 ⁻⁶ × Homogenate radioactivity (d.p.m.)		Unfractionated plasma membranes					
	¹⁴ C	³ H	¹⁴ C	³ H	Light plasma membranes		Heavy plasma membranes	
			¹⁴ C	³ H	¹⁴ C	³ H	¹⁴ C	³ H
1.5	8.2	55	9.7 (1.18)	26.0 (0.5)	1.26 (0.15)	3.91 (0.07)	6.25 (0.76)	16.6 (0.30)
2.25	5.5	49	17 (3.16)	46.0 (1.1)	3.94 (0.7)	11.3 (0.23)	9.6 (1.75)	25.0 (0.51)
6	2.85	32	12 (4.2)	31.6 (1.0)	1.82 (0.65)	5.45 (0.17)	6.9 (2.4)	18.0 (0.57)

are in agreement with those of Ray, Lieberman & Lansing (1968), are compatible with a precursor-product relationship between the rough- and smooth-endoplasmic reticulum and the plasma membrane, and suggest that liver plasma-membrane protein components are derived from precursors synthesized in the endoplasmic reticulum, presumably by the membrane-bound ribosomes. Further, the extensive differences in composition observed between the liver intracellular and surface membranes raise the possibility of the existence of a population of membrane-bound ribosomes concerned mainly with the synthesis of plasma-membrane proteins. Such a topological segregation of different populations of ribosomes has been suggested to explain the developmental changes

occurring in metamorphosing cells (Tata, 1970). A similar suggestion of the presence of two kinds of polyribosomes has also been put forward to explain the logistics of the synthesis of exported and retained proteins in differentiated animal cells by Campbell (1970).

The major reason for the present work was to compare the rates of synthesis of the plasma-membrane subfractions in the hope of finding the cellular origin of the vesicles of the light fraction. The enzymic, chemical and immunological markers show highest enrichment in the light fraction and on the basis of histochemical evidence (Novikoff, Essner, Goldfischer & Heus, 1962) it was suggested that the bile canalicular region of the liver parenchyma cell is the source of this fraction (Evans,

Fig. 5. Incorporation profiles of ^3H (●) and ^{14}C (○) radioactivity in (a) rough-microsomal, (b) smooth-microsomal, (c) light-plasma membrane and (d) heavy-plasma membrane fractions. Labelled components were separated by electrophoresis in polyacrylamide gels and radioactivity was determined as described in the Experimental section. The membrane fractions were isolated 4 h after injection of isotopes.

1970a). Thines-Sempoux, Amar-Costesec, Beaufay & Berthet (1969) isolated from rat liver microsomes a subfraction that possessed many properties in common with the light-plasma membrane subfraction studied in the present paper, e.g. high cholesterol content and high 5'-nucleotidase and alkaline phosphodiesterase specific activities. The authors suggested that this microsomal subfraction could represent a specialized area of the endoplasmic reticular network, or that it could be a biosynthetic precursor, or derivative of the plasma membrane of the cell. Another possible origin of the plasma-membrane light fraction is suggested by morphological and cytochemical studies of intact livers that showed the presence of pinocytotic vesicles, which fused together to form larger vacuoles, which in turn were observed to move into the cytoplasm (Graham, Limpert & Kellermeyer, 1969; Claude, 1970). Finally, the similarity of the median densities in sucrose gradients suggest that

the light fraction may originate from elements of the Golgi apparatus. However, the absence of glycosyltransferase activity from the light fraction eliminates this last possibility. The results presented now clarify from a biosynthetic standpoint the status of the light-plasma membrane subfraction. The rates of incorporation of both protein and carbohydrate precursors into the light subfraction and the unfractionated plasma membrane are similar. These results are consistent with the view that the vesicles of the light fraction are derived from the plasma membrane and eliminate the possibility that they are a biosynthetic precursor.

Addition of the sugar residues of liver glycoprotein is generally agreed to occur in the Golgi apparatus which is a component of the smooth-microsomal fraction (Schachter *et al.* 1970; Fleischer & Fleischer, 1970). The localization of UDP-*N*-acetylglucosamine transferase and the high

specific radioactivity of glucosamine in the smooth-microsomal fraction are in agreement with this location. The minor peak of glucosamine incorporation into the rough-microsomal fraction at 30 min may reflect the glycosyltransferase activity demonstrated in this fraction and also reported by others (Lawford & Schachter, 1966; Hallinan, Murty & Grant, 1968). The parallel rates of incorporation of [^{14}C]glucosamine and [^3H]leucine into the plasma-membrane fractions suggest that membrane proteins and glycoproteins are synthesized in a co-ordinated fashion. Similar results on the metabolism of glucosamine by rat liver were obtained by Kawasaki & Yamashina (1971) who assumed that incorporation occurred solely into the membrane glycoproteins. However, it is now shown that plasma-membrane glycolipids account for an appreciable proportion of the incorporated radioactivity. The relatively rapid labelling of the glycolipids, especially in the light fraction, points to an important metabolic role for these plasma-membrane components.

The different rates of synthesis of membrane glycoproteins and glycolipids may indicate that glycolipids are incorporated into the plasma membrane independently of newly synthesized membrane protein components. This interpretation is supported by other studies on the synthesis and turnover of liver microsomal lipids and proteins (Omura, Siekevitz & Palade, 1967; Widnell & Siekevitz, 1967). However, Bosmann, Hagopian & Eylar (1969) found parallel rates of [^{14}C]glucosamine incorporation into the plasma-membrane glycolipids and glycoproteins of HeLa cells and interpreted their results as favouring the initial formation of lipoprotein subunits that are subsequently incorporated into the plasma membrane.

The well-established heterogeneity of the liver microsomal fraction (Dallman, Dallner, Bergstrand & Ernster, 1969; Glaumann & Dallner, 1970; Amar-Costesec, Beaufay, Feytmans, Thines-Sempoux & Berthet, 1969) can be partly accounted for by the presence in it of the two plasma-membrane fractions studied in the present paper. The incorporation profiles of the microsomal and plasma-membrane fractions differ greatly. The present results enable a better delineation of the mechanism that elaborates the extensive membrane network of the liver parenchymal cell. Examination of the incorporation into individual proteins and glycoprotein components separated by polyacrylamide-gel electrophoresis indicated considerable heterogeneity at the molecular level. Further studies utilizing plasma-membrane fractions of relatively simple composition prepared by using pyridine and detergent fractionation procedures (Gurd *et al.* 1971) are expected to yield further information on the

mechanism of biosynthesis of the plasma-membrane components.

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