SELECTIVE RELEASE OF CONTENT FROM MICROSOMAL VESICLES WITHOUT MEMBRANE DISASSEMBLY

II. Electrophoretic and Immunological Characterization of Microsomal Subfractions

GERT KREIBICH and DAVID D. SABATINI

From the Department of Cell Biology, New York University School of Medicine, New York 10016

ABSTRACT

Rough and smooth microsomes were shown to have similar sets of polypeptide chains except for the proteins of ribosomes bound to the rough endoplasmic reticulum (ER). More than 50 species of polypeptides were detected by acrylamide gel electrophoresis, ranging in molecular weight from 10,000 to approximately 200,000 daltons. The content of rough and smooth microsomes was separated from the membrane vesicles using sublytic concentrations of detergents and differential centrifugation. A specific subset of proteins which consisted of approximately 25 polypeptides was characteristic of the microsomal content. Some of these proteins showed high rates of in vivo incorporation of radioactive leucine or glucosamine, but several others incorporated only low levels of radioactivity within short labeling intervals and appeared to be long-term residents of the lumen of the ER. Seven polypeptides in the content subfractions, including serum albumin, contained almost 50% of the leucine radioactivity incorporated during 5 min and cross-reacted with antiserum against rat serum. Almost all microsomal glycoproteins were at least partly released with the microsomal content.

Smooth microsomes contained higher levels of albumin than rough microsomes, but after short times of labeling with [^{3}H]leucine the specific activity of albumin in the latter was higher, supporting the notion that newly synthesized serum proteins are transferred from rough to smooth portions of the ER. On the other hand, after labeling for 30 min with [^{3}H]glucosamine, smooth microsomes contained higher levels of radioactivity than rough microsomes. This would be expected if glycosidation of newly synthesized polypeptides proceeds during their transit through ER cisternae.

The labeling pattern of membrane proteins in microsomes obtained from animals which received three daily injections of [^{3}H]leucine, the last administered 1 day before sacrifice, followed the intensity of bands stained with Coomassie blue,

with a main radioactive peak corresponding to cytochrome P 450. After the long-term labeling procedure most content proteins had low levels of radioactivity; this was especially true of serum proteins which were highly labeled after 30 min.

INTRODUCTION

The lumen of endoplasmic reticulum (ER) cisternae is an important subcellular compartment, the properties of which are yet largely unknown. In liver cells, secretory proteins destined for the bloodstream are thought to be manufactured in membrane-bound polysomes and to be directly discharged into the ER cisternae (Palade and Siekevitz, 1956; Campbell et al., 1960; Peters, 1962 *a*,*b*; Redman and Sabatini, 1966; Redman, 1969; Ganoza and Williams, 1969), where they may be modified by enzymes bound to the limiting membranes (Molnar et al., 1965; DeLorenzo et al., 1966; Wagner and Cynkin, 1971; Redman and Cherian, 1972), before being transferred to the Golgi apparatus (Glaumann, 1970; Glaumann and Ericsson, 1970; Schachter et al., 1970; Peters et al., 1972). The intracisternal route provided by the ER may also be utilized by proteins retained intracellularly which are diverted from the secretory pathway into other membrane-bound compartments, such as lysosomes (de Duve and Wattiaux, 1966; Goldstone and Koenig, 1972), or peroxisomes (Higashi and Peters, 1963 *a*,*b*; Kashiwagi et al., 1971; but see Redman et al., 1972, and Lazarow and de Duve, 1973). It has also been shown that at least some membrane proteins of the ER are synthesized on membrane-bound ribosomes (Omura, 1973), and it is possible that these proteins are first discharged into the cisternal cavity before being incorporated into the membranes.

To understand the discriminating role which the ER exerts over the fate of proteins reaching the cisternal cavity, it will be necessary to characterize the molecular composition and properties of the intracisternal environment. Extensive fragmentation and resealing of ER membranes occurs during cell fractionation, leading to the formation of microsomal vesicles in which the cisternal content of the ER is preserved (Palade and Siekevitz, 1956). In a previous paper we have shown (Kreibich et al., 1973) that a protein subfraction which represents the vesicular content can be released from microsomes by low detergent concentrations. The levels of detergent needed for the release of content were insufficient to produce any extensive membrane disassembly or reorganization of membrane components measurable by solubilization of phospholipids, ribosome detachment, or loss of membrane proteins of the microsomal electron transport chains. It was physicochemically and electronmicroscopically demonstrated that low detergent concentrations produced the reversible formation of membrane openings in microsomes which may allow the leakage of content proteins (see also Ernster et al., 1962; Kuriyama, 1972; Weihing et al., 1972). The location of the releasable proteins within microsomal vesicles was confirmed through the demonstration that in intact microsomes the proteins were inaccessible to proteases (Kreibich et al., 1973), and to enzymatic iodination by lactoperoxidase (LPO), unless low detergent concentrations were present (Kreibich and Sabatini, 1973; Kreibich et al., 1974).

In this paper it is shown that the content of microsomes is a complex mixture of proteins and glycoproteins with different fates and metabolic stabilities. Proteins of rapid synthesis which are destined for secretion, such as albumin and other serum proteins, were identified in the microsomal content in addition to other proteins of rapid and slow synthesis which are retained intracellularly.

MATERIALS AND METHODS

Materials

Glycine and sodium dodecyl sulfate (SDS) were purchased from Sigma Chemical Co., St. Louis, Mo.; Butyl-PBD, methyl bisacrylamide, and *N*,*N*,*N'N'*-tetramethylethylenediamine (TEMED) from Eastman Kodak Co., Rochester, N.Y.; Coomassie brilliant blue from Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N.Y.; six-times recrystallized bovine serum albumin from Armour Pharmaceutical Co., Chicago, Ill.; Triton-X 100 from Rohm and Haas Co., Philadelphia, Pa.; rat liver catalase was kindly provided by Dr. P. Lazarow, The Rockefeller University, New York.

The molecular weights of proteins used for calibration of the SDS-disk acrylamide gels were those listed by Weber and Osborn (1969).

Composition of solutions: TKM, 50 mM Tris-HCl pH 7.5, 25 mM KCl, 5 mM $MgCl_2$; HSB, 50 mM Tris-HCl pH 7.5, 500 mM KCl, 5 mM $MgCl_2$; LSB, 50 mM Tris-HCl pH 7.5, 50 mM KCl, 5 mM $MgCl_2$. Sources of

other materials and analytical procedures were described in a previous paper (Kreibich et al., 1973). Conditions of centrifugation are given in an abbreviated form. For example, (60 min-50K-A321) indicates a 60-min centrifugation at 50,000 rpm in the A-321 rotor of the IEC B-60 centrifuge (International Equipment Company, Needham Heights, Mass.). Approximately equivalent conditions were chosen when Spinco centrifuges (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) were used (see also Adelman et al., 1973 *a*).

Labeling and Subfractionation of RM and SM

Rough microsomes (RM), and smooth microsomes (SM), containing [^{3}H]- and [^{14}C]leucine-labeled proteins and [^{3}H]glucosamine-labeled glycoproteins were prepared according to Adelman et al., (1973 *a*) after in vivo administration of radioactive precursors. The procedures for in vivo labeling and the specific activities obtained in the cell fractions are the same as specified in Tables I and II of a previous paper (Kreibich et al., 1973). For the experiments combining autoradiography with immunodiffusion or with immunoelectrophoresis, microsomes were obtained from rats sacrificed 30 min after injection of L-[U-^{14}C]leucine (25 μCi/g body weight; specific activity 280 mCi/mmol; New England Nuclear, Boston, Mass.).

Rough microsomes were stripped of ribosomes by the puromycin-KCl procedure of Adelman et al. (1973 *b*). The stripped RM (RMstr) were recovered by differential centrifugation (20 min-30K-Ti60) at 20°C.

For subfractionation, microsomes were washed once in HSB (20 min-20K-A211) and resuspended in 1–3 ml of LSB (3–6 mg protein/ml). Detergents (sodium deoxycholate [DOC] or Triton X-100) were added at the concentrations indicated in the text. After incubation for 30 min at 0°C, soluble and sedimentable subfractions were separated by differential centrifugation (60 min-50K-A321) at 3°C.

Microsomal suspensions were also subfractionated after sonication in an ice-bath using a Branson sonifier (Heat Systems, Inc., Plainview, N. Y.) equipped with a microtip. Pulses of 10 s were given with an intensity reading of 2 A (setting no. 4) and 20-s intervals for cooling. Foaming was avoided. Alternatively, 1–5-ml samples were disrupted in an Aminco pressure cell (American Instrument Co., Inc., Travenol Laboratories, Inc., Silver Spring, Md.) by decompression from 4,000 lb/in^2. Content and membranes were separated by centrifugation (90 min-40K-Ti60).

For the immunoprecipitation experiments with microsomal subfractions, Triton X-100 (see Kreibich and Sabatini, 1974) was used since this nonionic detergent produces less unspecific precipitation with antisera than DOC. Sedimentable membrane fractions obtained after 0.15% Triton X-100 were resuspended in the original volume of LSB containing 1% Triton X-100. Ribosomes and insoluble aggregates were removed by centrifugation (90 min-40K-Ti60), before addition of antiserum.

Electrophoresis

Aliquots (0.4 ml containing 0.5–6 mg protein/ml) of microsomal suspensions, supernates, or sedimentable subfractions resuspended in the original volume of water received 0.1 ml of the spacer gel buffer (Table I in Maizel, 1971), 50 μl 25% SDS, 0.1 ml glycerol, 5 μl mercaptoethanol, and 1 drop of a bromophenol blue solution (0.1%), and were heated to 100°C for 2 min. Samples (10–200 μl) containing about 10 μg of a single standard protein or up to 400 μg of total RM proteins were loaded into the slots. In some cases, samples which were too diluted received 0.1% DOC as a carrier to aid in precipitation with 10% trichloroacetic acid (TCA), (30 min at 0°C). TCA precipitates were resuspended in water and neutralized with NaOH before processing as described above.

SDS polyacrylamide gel electrophoresis was performed essentially according to the procedure for the SDS-disk system (page 188, Table I in Maizel, 1971). Vertical electrophoresis cells (E-C Apparatus, St. Petersburg, Fla.) were used with slabs 3 or 6 mm in thickness. The gels shown in the figures are 7.5%, 10%, or 12% acrylamide with a methylene bisacrylamide concentration of 0.2–0.36%.

Linear gradient gels ranging from 7 to 12% acrylamide were prepared in a slab gel apparatus. Polymerization in the gradient former was avoided by reducing the ammonium persulfate concentration to 0.025%. The stacking gel contained 4% acrylamide. The molecular weight range covered by these types of acrylamide gels extends from ~10,000 to >300,000 Daltons.

Electrophoresis at constant current (15–60 mA) was run for about 12–18 h at room temperature without diluting or exchanging the buffer in the electrode compartments. Runs were stopped when the front (marked by bromophenol blue or pyronin Y) was 13–20 cm within the resolving gel.

Slab gels were stained while shaking in a tray with 300–500 ml of 0.2% Coomassie brilliant blue R250 in methanol-water (1:1 vol/vol) containing 7% acetic acid. The staining solution was changed twice within 24 h and excess stain was removed with acetic acid-methanol-water (7:20:73 vol/vol). Gels were photographed with a yellow or orange filter.

For staining of glycoproteins (PAS-stain) the procedure of Fairbanks et al. (1971) was followed, but incubation times were doubled for slab gels 6 mm in thickness.

Radioactivity Distribution within the Gels

Stained 6-mm slab gels were cut into longitudinal strips, which were sliced (88 slices per 100-mm length) with a device consisting of parallel steel wires. Slices were

transferred to scintillation vials which were incubated at 50°C for 48 h with 0.5 ml 20% H_2O_2 and counted with 8 ml of scintillation fluid (8 g Butyl-PBD and 80 g naphthalene in 1 liter Dioxane) in a Beckman model LS250 (Beckman Instruments, Inc., Fullerton, Calif.) scintillation counter.

Immunodiffusion, Immunoelectrophoresis, and Autoradiography

Pattern D immunodiffusion plates were purchased from Hyland Div., Travenol Laboratories Inc., Costa Mesa, Calif. Immunoelectrophoresis on agar-coated slides (5% Noble agar in 0.012 M Tris barbital buffer pH 8.8), with 0.05 M Tris barbital pH 8.8 as electrode buffer, was run for 2 h in a Gelman Chamber (Gelman Instrument Co., Ann Arbor, Mich.). Rabbit serum and rat serum were prepared from blood obtained by heart puncture. Rabbit antirat serum was purchased from Miles Laboratories, Inc., Miles Research Div., Kankakee, Ill. Precipitation bands in agar films were stained with amido black (0.5%) in 5% acetic acid and 5% glycerol. X-ray film (Royal X-Omat, Eastman Kodak Co.), was used for autoradiography.

Immunoprecipitation of Serum Proteins in Microsomal Subfractions

Labeled microsomes were prepared from animals which received [^{3}H]leucine for 5 min in vivo. Table I gives the details of the labeling procedure and the distribution of TCA-insoluble radioactivity in the microsomal subfractions obtained by 0.15% Triton X-100 treatment. Soluble subfractions or sedimentable membranes solubilized in 1% Triton X-100 were concentrated eightfold with a model 8MC Amicon ultrafiltration apparatus, (Amicon Corp., Lexington, Mass.), using the PM10 filter. Less than 3% of the total radioactivity was lost in the ultrafiltrates. All sera and microsomal subfractions were preincubated separately for 1 h at 37°C and centrifuged (30 min-20K-no. 40) before use to remove aggregates which contained less than 2% of the total radioactivity.

For immunoprecipitation 150 μl of the concentrates were incubated with 150 μl of rabbit antirat serum (Miles Laboratories), or serum from nonimmunized rabbits for 1 h at 37°C and 48 h at 4°C. The amount of antiserum used was in slight excess and was sufficient to precipitate all the antigen present as demonstrated by analysis of the supernates on Ouchterlony plates by diffusion against rabbit antiserum or rat serum.

The precipitates were washed twice with buffered saline (0.01 M Tris-HCl, pH 7.8) to remove radioactivity carried down unspecifically. Less than 2–3% of the radioactivity in precipitates from microsomal content (0.15% Triton X-100) and less than 7% of the radioactivity in precipitates from solubilized membranes (1% Triton X-100) was removed in these washes. These values were added to the soluble radioactivity.

Supernates were directly processed for SDS-disk

TABLE I

*Distribution of Newly Synthesized Proteins in Microsomal Subfractions from Rat Liver**

	RM‡			SM‡		
	Recovery			Recovery		
	Protein	Radio-activity	dpm/mg × 10^{-5}	Protein	Radio-activity	dpm/mg × 10^{-5}
Total microsomes	100% (4.6 mg)	100%	3.56	100% (4.2 mg)	100%	2.74
Released subfractions (0.15% Triton X-100)	22%	53%	8.98	26%	64%	6.71
Sedimentable subfraction solubilized in 1% Triton X-100	37%	14%	1.35	43%	11%	0.73
Residue and ribosomes	41%	33%	2.90	31%	25%	2.2

* L-[4,5-^{3}H]Leucine (New England Nuclear, Boston, Mass., specific activity 67.6 Ci/mmol) was injected (1 μCi per gram body weight) into the portal vein of rats which were sacrificed after 5 min. The livers (22 g) from three rats (~250 g each) were homogenized, and RM and SM were prepared as described by Adelman et al., (1973 *a*). No TCA-precipitable radioactivity was found in rat serum. Released and sedimentable subfractions were prepared after 0.15% Triton X-100 treatment. Sedimentable membranes were recovered and dissolved in 1% Triton X-100. After differential centrifugation, a solubilized membrane fraction and a residual pellet containing ribosomes were obtained.
‡ RM and SM derived from 1 g of liver contained approximately 7 mg protein each.

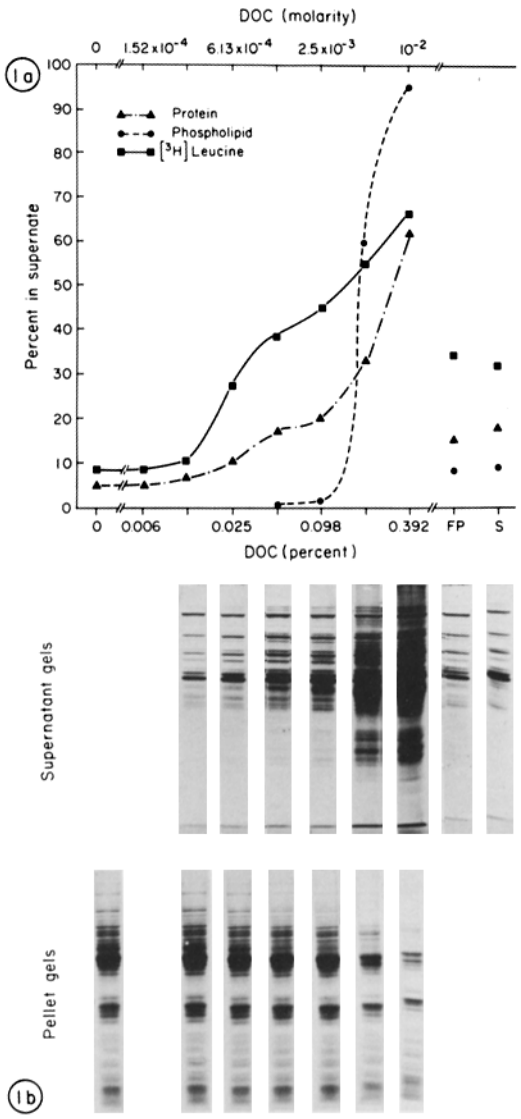

FIGURE 1 Selective release of proteins from RM after detergent, sonication, or French press treatment. RM ([³H]leucine, 30 min) were resuspended (4 mg protein/ml) in a modified LSB (10 mM Tris). Aliquots (1 ml) were treated with a series of DOC concentrations, sonicated (6 × 10 s), or passed twice through the French press (Kreibich et al., 1973). Supernates and pellets were obtained by differential centrifugation (60 min-60K-A321). Pellets were resuspended in an amount of water equivalent to the supernate. (*a*) Distribution of TCA-precipitable radioactivity (—■—), total protein (-·-▲-·-), and phospholipid (--■--) in supernates. For each sample, the sum of radioactivity, phospholipid, or total protein in both subfractions was taken as 100%. (*b*) SDS acrylamide (10%) gel electrophoresis of proteins in supernates and resuspended sediments (pellets). 200 μl of supernate processed for electrophoresis (see Materials and Methods), or 100 μl of processed sediment, both reduced with mercaptoethanol, were loaded on to slab gels (6-mm thickness). Photographs of gel strips from supernates and pellets were aligned under the corresponding points representing the release of microsomal constituents.

acrylamide electrophoresis. Immunoprecipitates were dissolved in 300 μl of "sample buffer" containing 2% SDS. Radioactivity was determined in aliquots of the supernates, in the redissolved immunoprecipitates, and in the washings after addition of Nuclear Chicago Solubilizer (NCS, Amersham/Searle Corp., Arlington Heights, Ill.), and a toluene scintillator.

RESULTS

A. Electrophoretic Characterization of Proteins released from Microsomes

A characteristic set of microsomal proteins (polypeptide bands in supernatant gels in Fig. 1 *b*) was selectively released from microsomes treated with DOC at concentrations which were insufficient to dissolve membrane phospholipids (>0.098% DOC, Fig. 1 *a* --●--●--). The set of proteins easily released by detergent contained mainly proteins of high molecular weight (bands in the top third region of the supernatant gels in Fig. 1 *b* and Fig. 3) which were also released by mechanical disruption in the French press or by sonication (supernatant gels, Fig. 1 *b*, FP and S). These proteins represented less than 20% of the total protein in microsomes (Fig. 1 *a* -·-▲-·-▲-·-), but contained a major fraction of the newly synthesized polypeptides labeled in vivo for 30 min with [³H]leucine (Fig. 1 *a* —■—■—) (see also Kreibich et al., 1973).

From 0.012 to 0.098% DOC (Fig. 1 *b*), only the intensity of the released protein bands increased with the level of detergent. On the other hand, at DOC concentrations higher than 0.098%, which produced phospholipid solubilization (Fig. 1 *A*), the composition of the released protein set changed strikingly. Numerous protein bands appeared in supernatant gels which at lower DOC concentrations or after mechanical disruption were present only in the sediments.

Since bound ribosomes remain sedimentable even after treatment of microsomes with high DOC concentrations, a set of bands with the mobility of ribosomal proteins (most of which are smaller than 40,000 Daltons) was regularly found in the

lower half of all sediment gels (Fig. 1 *b*, see also Fig. 2 and Fig. 3 *c*). There were, however, microsomal proteins (bands in the upper part of last pellet gel in Fig. 1 *b*) which either because of aggregation or of specific attachment to ribosomes also remained sedimentable at high DOC concentrations (e.g., 0.39% DOC).

Several microsomal membrane proteins of low molecular weight were difficult to identify because they overlapped in mobility with proteins of bound ribosomes. These proteins were reocgnized by comparing gradient gels (7–12% in acrylamide) of RM obtained before (Fig. 2 *a*) and after (Fig. 2 *b*) ribosomes were detached, using a combination of puromycin and a high salt buffer (Adelman et al., 1973 *b*). The low molecular weight membrane proteins are represented by those bands *not corresponding* to arrows in Fig. 2. They were identified as corresponding to true membrane proteins since they were not released by the low detergent procedure (Fig. 1 *b*).

Fig. 4 (lower part) schematically represents the patterns of protein bands obtained by SDS acrylamide (7.5%) gel electrophoresis from supernatant and sedimentable subfractions prepared from RM by low detergent concentration treatment (0.049% DOC) (cf. Fig. 3). The drawings serve to introduce a numbering system which will be used to designate the individual bands. Table II also lists all the microsomal proteins recognized as individual bands in subfractions obtained by the low detergent procedure and analyzed in 7.5 and 10% gels. Approximate molecular weights were assigned using calibration curves.[1]

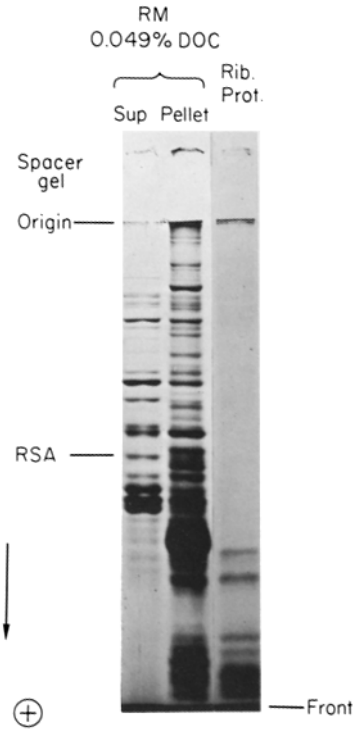

FIGURE 3 Electrophoretograms of microsomal subfractions obtained from RM, and of ribosomal proteins from free polysomes. Supernates and pellets from rough microsomes treated with 0.049% DOC were processed for electrophoresis as described in Materials and Methods. Rat liver free polysomes obtained by the procedure of Adelman et al. (1973 *a*) were washed in HSB containing 0.5% DOC, resuspended in water (2 mg/ml), and similarly processed. Aliquots from microsomal subfractions (100 μl) and polysomes (50 μl) were loaded on 7.5% acrylamide gel slabs 6 mm thick.

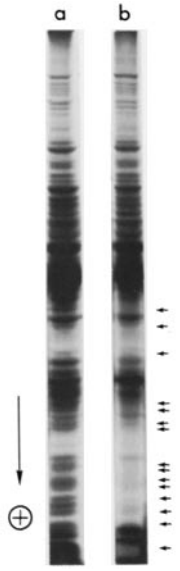

FIGURE 2 Gradient gel electrophoresis of RM, and of RM stripped of ribosomes (RMstr) by the puromycin KCl procedure. 100 μl of processed RM (*a*) and RMstr (*b*) containing about 150 μg protein each was loaded onto a slab gradient gel (7–12% acrylamide, 3 mm thick, 23 cm long). Arrows indicate bands of ribosomal proteins which are lost from RM after the stripping of ribosomes.

[1] It may be noted that, as reported previously by Weber and Osborn (1969) and Neville (1971), there is a not strictly linear relationship between the mobility of proteins in acrylamide gels expressed as Rf, and the log of their molecular weight.

B. Kinetics of Labeling of Microsomal Proteins

To correlate the kinetics of synthesis of microsomal proteins with their distribution after detergent treatment, subfractions prepared from microsomes containing proteins and glycoproteins labeled in vivo with [^{3}H]leucine and [^{3}H]glucosamine (preparations listed in Table I of Kreibich et al., 1973), were analyzed in SDS acrylamide gels which after staining were sliced to determine the radioactivity of individual bands. To ensure that sufficiently high levels of radioactivity were analyzed, aliquots which contained high amounts of protein (up to 400 μg) were loaded on to 6-mm thick gels (7.5%). By recording the correspondence between bands in gel photographs and sliced fractions, it was possible to obtain a rather precise correlation between the patterns of staining and the radioactivity distribution. It thus became clear that in supernates from short-term-labeled rough microsomes ([^{3}H]leucine, 30 min) (Fig. 5), band no. 33, which was a minor component in the staining pattern, was in exact correspondence with the major species of recently synthesized labeled proteins. It will be later shown (see section *D*) that this band corresponds in mobility to rat serum albumin (RSA) with which it also cross-reacts immunologically. Many other protein bands (see Table II and Fig. 4), nos. 10–13, 21, 30, 39, 40, and 42 in the set of released proteins were also radioactive (Fig. 5) and were therefore synthesized within the 30-min labeling interval. The distribution of [^{3}H]leucine radioactivity also showed that several released proteins were not highly labeled during the 30-min interval (see Table II).

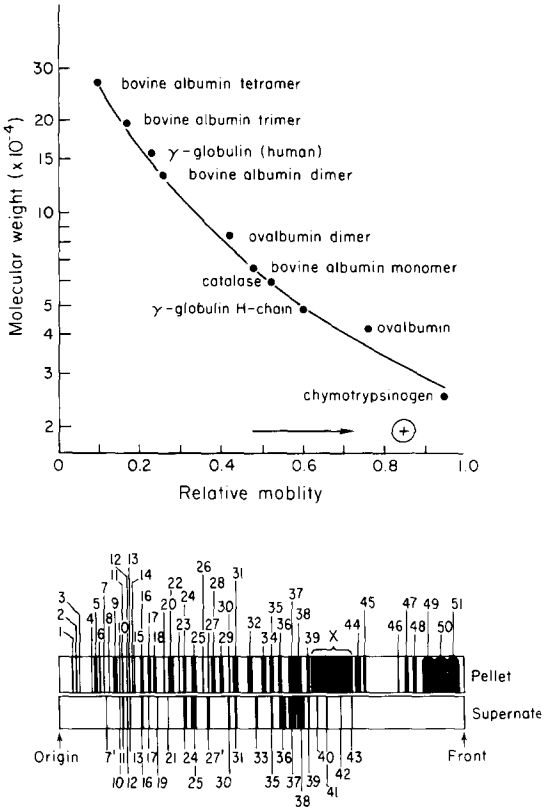

FIGURE 4 (upper part) Calibration curve derived from the mobility of markers used for the approximate determination of molecular weights of proteins in 7.5% acrylamide gels. (lower part) Schematic representation of the staining pattern of gels from supernatant and sediment subfractions of rough microsomes treated with 0.049% DOC (see Fig. 3). Bands have been numbered in the order of increasing mobility. The scheme has been drawn so that the position of the bands corresponds to their relative mobility in the calibration curve for molecular weights. Some bands are present in gels of both subfractions. Bands numbered 7′ and 27′ are probably different from 7 and 27.

A comparison of the distribution of labeled proteins in microsomal subfractions was made, taking into account the different amounts of protein loaded into gels. This showed that most labeled proteins were very extensively released by the low DOC concentration treatment, and that in the case of the protein represented by band no. 33, the release was approximately 80% complete in 0.049% DOC. For other labeled proteins (e.g. nos. 10–13), however, a larger fraction (~50%) of the radioactivity was found with the sediment. A radioactive component which always remained associated with the sedimentable membranes after this treatment migrated to a position coinciding with that of the major membrane band labeled X in Fig. 4 and Table II. Band X corresponds in mobility and intensity with the component identified by other authors as cytochrome P 450 (Dehlinger and Schimke, 1971 and 1972; Alvares and Siekevitz, 1973). However, two other protein bands (nos. 39 and 40) in the superantant fractions had similar mobilities to X. For this reason it could not be established if the radioactivity at the level of X in the sedimentable subfraction represents incomplete release of components 39 and 40 from the sedimentable membranes, or true incorporation into cytochrome P 450. Because of the large amount of cytochrome P 450 which is present

TABLE II

Characterization of Proteins in Subfractions of RM obtained by Detergent Treatment

Protein numbers		Molecular weight Dalton $\times 10^{-3}$	L-[^{3}H]leucine incorporated in				D-[^{3}H]glucosamine incorporated in 30 min		PAS stain	Precipitated with antirat serum	Relative mobility of
			30 min		2 min						
Pellet	Supernate		RM	SM	RM	SM	RM	SM			
1	—						+	+			
2	—						+	+			
3	—						+	+			
4	—	>215									
5	—										
6	—										
7	7										
8	—										
9	—	215									
10	10		+	+	+	+	+	+	+	+	
11	11	200	+	+	+	+	+	+	+	+	RSGP
12	12		+	+			+	+	+	+	
13	13	190	+	+			+	+	+	+	
14	—										
15	—										
16	16	160							+	+	RSGP
17	17								+		RSGP
18	—										
—	19	140						+			
20	—										
—	21		+	+	+	+					RSGP
22	—	130									
23	—										
24	24	110									
25	25	105						+	+		RSGP
26	—										
27	27	92						+			
28	—										
29	—										
30	30	78	+	+	+	+	+	+	+	+	RSGP
31	31							+			
32	—	70									
—	33	67	+	+	+	+				+	RSA
34	—								+		
35	35	60					+	+	+		RSGP; catalase
36	36	56									
37	37	54					+	+	+		
38	38	51			+	+		+			
39	39		+	+	+	+	+	+	+		RSGP
X	—										P450
?	40	49	+	+	+	+			+		
?	41	47									
?	42	44	+	+	+	+	+	+	+		
?	43	42									
44	—	40									RP
45	—								+		
46	—	34									
47	—	32					+	+			RP

TABLE II—*Continued*

Protein numbers		Molecular weight	L-[^{3}H]leucine incorporated in				D-[^{3}H]glucosamine incorporated in 30 min				
			30 min		2 min						
Pellet	Supernate	Dalton $\times 10^{-3}$	RM	SM	RM	SM	RM	SM	PAS stain	Precipitated with antirat serum	Relative mobility of
48											RP
49	—	31							+		
50	—										RP
51	—	25									RP

Abbreviations: RSA, rat serum albumin; RSGP, bands which correspond in mobility to rat serum glycoproteins shown in Fig. 9; RP, bands which correspond to ribosomal proteins shown in Fig. 5. The protein numbers correspond to those in Fig. 4.

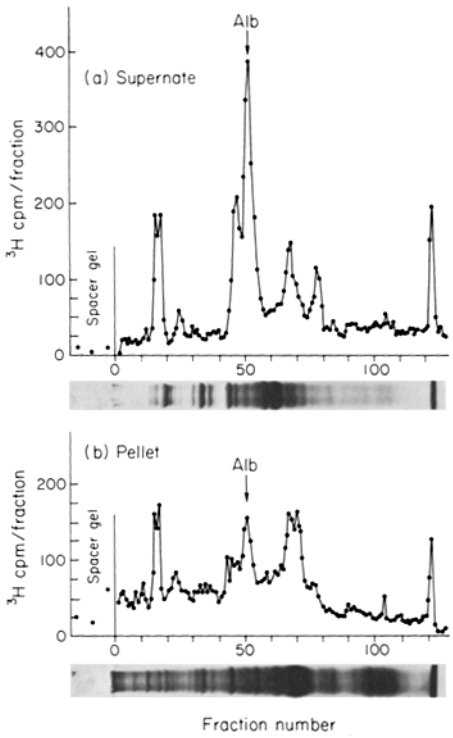

FIGURE 5 Distribution of short-term labeled proteins in microsomal subfractions from rough microsomes, analyzed by acrylamide (7.5%) gel electrophoresis. RM containing short-term labeled proteins ([^{3}H]leucine, 30 min) were resuspended in LSB (5–6 mg protein/ml) and treated with 0.049% DOC. Supernate and pellet fractions were prepared and processed for electrophoresis. 200 μl of the supernate and 100 μl of sediment containing ~120 and 350 μg of protein, respectively, were loaded in each slot of 6-mm thick gels. The radioactivity distribution was determined after slicing the gels as described in Materials and Methods.

Photographs of stained gels before slicing and plots of the radioactivity distribution in each gel strip were aligned so that radioactivity peaks can be related with the corresponding stained bands.

in rat liver microsomes (Omura and Sato, 1964) and its relatively high rate of turnover (Dehlinger and Schimke, 1971); significant labeling of this protein may be expected even after a short time of in vivo incorporation.

It has previously been shown that rough and smooth microsomal membranes contain similar sets of proteins (Zahler et al., 1970; Hinman and Phillips, 1970; Dehlinger and Schimke, 1971). Electrophoretic patterns from sedimentable subfractions of SM differed from those of RM only in the region of ribosomal proteins, which were absent or poorly represented in smooth microsomes (cf. gel patterns in Figs. 5 and 6). Similar sets of releasable proteins were also obtained when RM and SM were treated with low detergent concentrations (0.049% DOC). However, for equal protein loads, band no. 33 (which corresponds to RSA), was always more intense and prominent in gels of supernates from SM. The labeling patterns of rapidly synthesized proteins in subfractions of rough and smooth microsomes obtained after 30 min of labeling with [^{3}H]leucine were also similar. In correspondence with the higher albumin concentration (Peters et al., 1971) for equal protein loads the radioactivity peak corresponding to band no. 33 (RSA) was usually greater in SM than in RM.

It is known (Glaumann and Ericsson, 1970; Peters et al., 1971; see also Table II in Kreibich et al., 1973), that secretion of labeled proteins into the blood begins approximately 15 min after [^{3}H]leucine administration. Shorter times of labeling are therefore needed to examine possible differences in the quality of newly synthesized

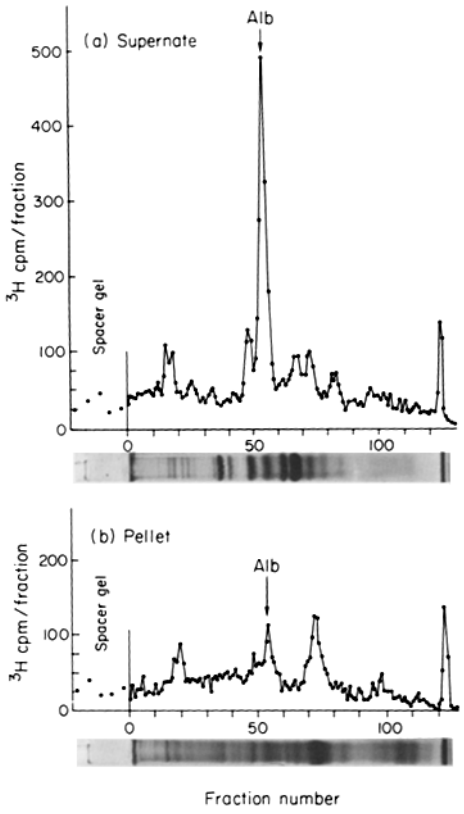

FIGURE 6 Distribution of short-term labeled proteins in microsomal subfractions from smooth microsomes analyzed by acrylamide gel electrophoresis. SM containing short-term labeled proteins ([^{3}H]leucine, 30 min) were treated with 0.049% DOC, processed, and analyzed as described for RM in the legend to Fig. 5.

polypeptides in rough and smooth microsomes. A 30-min labeling interval may also result in significant labeling of microsomal proteins which are membrane constituents. For these reasons subfractions of RM and SM obtained after a 2-min pulse of in vivo labeling were also analyzed electrophoretically for the radioactivity distribution. No labeled proteins were yet detected in serum but SM already contained considerable radioactivity (Table II of Kreibich et al., 1973). Nascent polypeptides of membrane-bound ribosomes recovered after total dissolution of membranes with 1% DOC contained ~50% of the total radioactivity in RM. Because of the heterogeneous size of the peptidyl-tRNA molecules, gels of sedimentable subfractions prepared from rough microsomes labeled in vivo for 2 min (Fig. 7) had a higher background of radioactivity than corresponding gels from SM (not shown). Nevertheless the labeling patterns of proteins in released subfractions were similar for rough and smooth microsomes. Such patterns were also similar to those from microsomes labeled for 30 min, with the exception that in supernatant subfractions from SM, the radioactivity peak corresponding to protein band no. 33 (RSA) was not as prominent at 2 min (not shown) as after 30 min (Fig. 6).

The distribution of proteins synthesized at lower rates than secretory proteins was examined in microsomal subfractions obtained from rats which received three daily injections of [^{14}C]leucine, the last 1 day before sacrifice (long-term labeling procedure, Table I in Kreibich et al., 1973). As

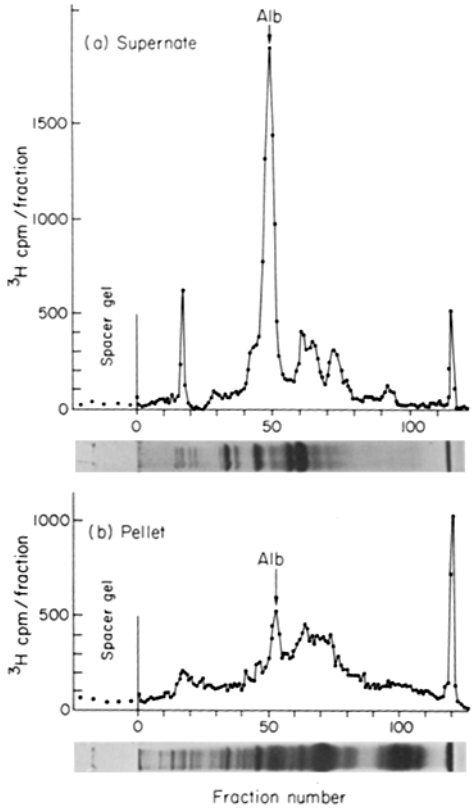

FIGURE 7 Distribution of pulse-labeled proteins in microsomal subfractions of rough microsomes analyzed by acrylamide gel electrophoresis. RM containing proteins and nascent polypeptides labeled during a short in vivo pulse of amino acid ([^{3}H]leucine, 2 min) were treated with 0.049% DOC, processed, and analyzed as described in the legend to Fig. 5.

expected, the labeling pattern of these proteins, most of which are likely to be membrane constituents (Fig. 8), was strikingly different from those of short-term (30 min) (Fig. 5) and pulse-labeled (2 min) microsomes (Fig. 7). After the long-term labeling procedure, the radioactivity distribution in gels of sedimentable and supernatant subfractions closely followed the intensity of staining of most bands. Proteins which were highly labeled after 2 and 30 min, and were released at low DOC concentrations, had almost negligible radioactivity after the long-term labeling procedure. A typical example was that of band no. 33 (RSA). On the other hand, major Coomassie blue bands in the residual microsomes which were not highly labeled after 30 min (such as band X and the ribosomal proteins), and small proteins which migrated together with the front (such as cytochrome b_5), were major radioactive components after the long-term labeling procedure.

C. Glycoproteins in Microsomal Subfractions and in Rat Serum

In rat liver the bulk of the protein-synthesizing activity of the rough endoplasmic reticulum is devoted to the manufacture of serum proteins (Peters et al., 1971; Redman and Cherian, 1972), several of which are known to be glycoproteins. It was therefore of interest to compare the electrophoretic pattern of serum proteins and glycoproteins with those of proteins and glycoproteins in microsomal subfractions prepared using different DOC concentrations. To this effect, SDS acrylamide gels of RM subfractions were first stained by the PAS method, photographed, and after extensive washing, counterstained for total proteins with Coomassie blue (Fig. 9). This double staining procedure allowed a very precise correlation between bands in both patterns. It was also possible to recognize glycoproteins which normally were not resolved as individual bands because their mobility coincided with that of major proteins which contained no carbohydrate. In rat serum, for example, two glycoproteins were masked by the prominent serum albumin band (Fig. 9, PAS, rat serum) and one glycoprotein in the sedimentable microsomal subfraction (Fig. 9, PAS, pellets) coincided with band X (cytochrome P 450). As expected (Fig. 9), most proteins in rat serum, with the major exception of albumin, were stained by the PAS method. The weak Coomassie blue band no. 33 in the released microsomal subfraction,

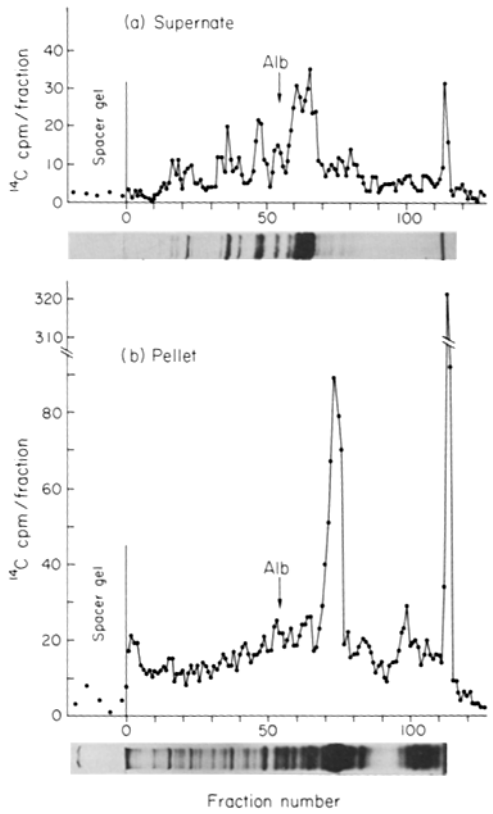

FIGURE 8 Distribution of long-term labeled proteins in RM subfractionated with 0.049% DOC. RM labeled by the long-term procedure ([^{14}C]leucine, 3 days) were treated with 0.049% DOC, fractionated, and processed as described in the legend to Fig. 5.

which was highly labeled after 30 min of in vivo [^{3}H]leucine incorporation, coincided in mobility with the RSA band in serum and was not stained by the PAS method. Ribosomal proteins in the sedimentable subfraction were also PAS negative.

It was surprising to find that the sets of PAS-positive microsomal glycoproteins in released and sedimentable subfractions obtained after DOC (0.025%) treatment were very similar. This indicates that contrary to the case of other proteins (e.g. no. 33), most glycoproteins were only partially released from microsomes by the low (0.025%) DOC concentration. One exception (lower half of PAS-stained pellet gels in Fig. 9) was a glycoprotein (no. 49) with an apparent molecular weight of 31,000 Daltons, which was exclusively found with the sedimentable membranes. Table II lists as presumptive serum glyco-

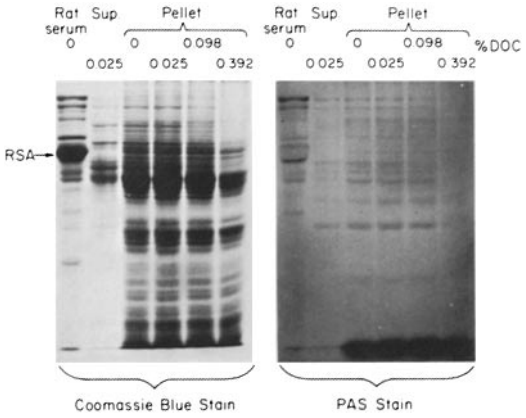

FIGURE 9 Patterns of PAS and Coomassie blue staining in gels of microsomal subfractions and rat serum, analyzed by SDS acrylamide (10%) electrophoresis. In this case, corresponding amounts of supernate and pellets were loaded in each slot, e.g. for 0.025% DOC, 70 μg of supernatant protein and 380 μg of protein from the sedimentable subfraction. Gels were stained by the PAS procedure, photographed, and counterstained with Coomassie blue.

proteins (RSGP) those PAS-positive bands in Fig. 9 which are represented at corresponding positions in gels of rat serum. However, some microsomal glycoproteins were not present in rat serum (nos. 34, 37, 42, 45, 49 of Table II), as may be expected for proteins which are not secreted into the bloodstream or must undergo further modification before secretion. Conversely, several serum glycoproteins (e.g., a serum protein in the 20,000 mol wt range) were not represented by corresponding bands in microsomal subfractions obtained from smooth or from rough microsomes. This of course, should be expected for at least those serum proteins which are of nonhepatic origin.

It was previously shown that after 30 min of [^{3}H]glucosamine administration, the smooth microsomal fraction contains per milligram of protein almost 10 times more glycoprotein radioactivity than rough microsomes (Kreibich et al., 1973; see also Schachter et al., 1970; Fleischer and Fleischer, 1970; Wagner and Cynkin, 1971; Sturgess et al., 1972; Redman and Cherian, 1972). In spite of this difference in the level of radioactivity, similar sets of labeled glycoproteins were found by acrylamide gel electrophoresis in RM, SM, and their subfractions (Fig. 10). As expected, band no. 33 (RSA) contained no [^{3}H]glucosamine radioactivity. Some glycoproteins which were released to a rather large extent, such as those represented by bands nos. 30, 35, and 39, were highly radioactive, but other highly labeled glycoproteins (nos. 10–13) were not as extensively released. Characteristically, sedimentable subfractions contained high levels of [^{3}H]glucosamine radioactivity (Fig. 10) in glycoproteins of a molecular weight larger than 200,000 (bands nos. 1, 2, and 3 of Table II and Fig. 4). These labeled proteins were found almost exclusively in the sedimentable subfractions and

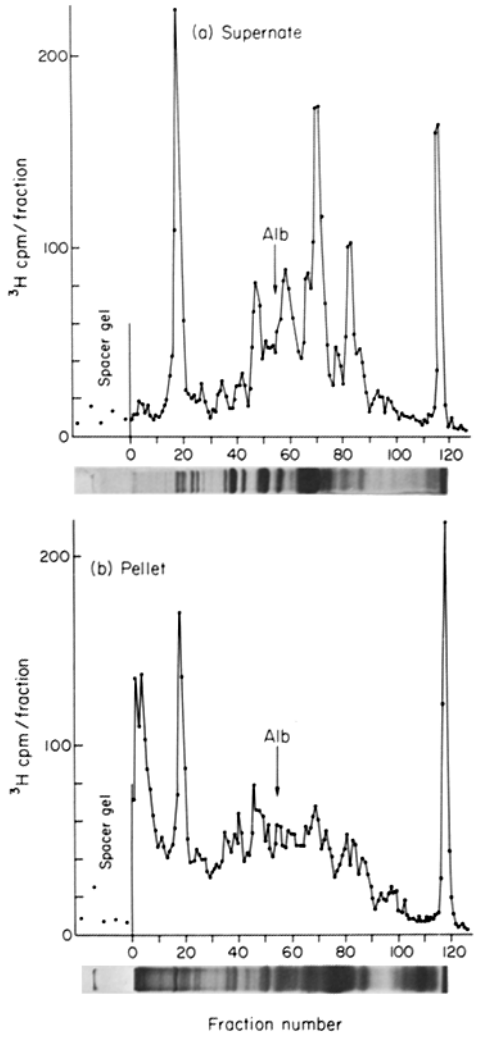

FIGURE 10 Distribution of radioactively labeled glycoproteins in microsomal subfractions analyzed by acrylamide gel electrophoresis. Smooth microsomes containing labeled glycoproteins ([^{3}H]glucosamine, 30 min) were treated with 0.049% DOC, and processed as described in the legend to Fig. 5.

therefore most probably represented true membrane components. Although these proteins were labeled with [^{3}H]glucosamine within 30 min, they showed no high incorporation of [^{3}H]leucine in the same interval (cf. Figs. 5, 6).

In gels of sedimentable subfractions, the PAS method intensely stained a material which migrated as a diffuse band, just ahead of the tracking dye. That region of the gel was not stained with Coomassie blue. It is likely that glycolipids and fatty aldehydes released from plasmalogens or peroxidated polyunsaturated fatty acids (see Fairbanks et al., 1971) migrate in this region.

D. Identification of Serum Proteins in the Released Microsomal Subfraction

The presumptive relationship of proteins in subfractions released from rough or smooth microsomes by low detergent concentrations to serum proteins was confirmed immunologically using antiserum against total rat serum. It was found (Table III) that approximately 50% of the radioactivity in polypeptides chains released from RM or SM labeled in vivo with [^{3}H]leucine for 5 min was present in an immunoprecipitate formed after incubation with rabbit antiserum against total rat serum. This fraction of radioactivity was greatly reduced to (~25%) when rat serum (~5 μl) was added to the released subfraction before the rabbit antiserum, showing that serum proteins were able to compete with the cross-reacting material released from the microsomes. On the other hand, control serum from a nonimmunized rabbit precipitated less than 2% of the radioactivity released by the low detergent concentration.

Immunoprecipitation tests were also carried out with sedimentable subfractions recovered by sedimentation after the low detergent treatment and solubilized in 1% Triton X-100. However, in this case only 15% of the total radioactivity from the dissolved membranes could be precipitated with antiserum against rat serum. At least part of this radioactivity was carried down unspecifically, since a precipitate containing 9% of the total radioactivity formed in samples incubated with control rabbit serum. Addition of rat serum to compete with any cross-reacting material instead of decreasing the amount of radioactivity precipitated, increased the nonspecific coprecipitation.

Specific immunoprecipitates obtained from released subfractions were solubilized, reduced, and analyzed by SDS acrylamide gel electrophoresis. Fig. 11 allows a comparison to be made of a typical Coomassie blue staining pattern obtained from the immunoprecipitate with patterns from rat serum and from the total released subfraction. It is clear that in addition to the most intensely stained heavy chain (H) band contributed by the immunoglobulin in the antiserum,[2] several other protein bands are present in the immunoprecipitate. These coincide in mobility with bands characteristic of the released pattern, bands nos. 30, 33, 16, and 10–13 in Table II. Of these, only bands 33, 30, and 16 are represented in rat serum; band no. 33 coincides in mobility with rat serum albumin. It is interesting that although proteins in bands 10–13 cross-react with serum proteins, proteins of the same mobility are not detected in serum. This raises the possibility that proteins nos. 10–13 are precursors of serum proteins which are modified before secretion into the bloodstream. It is also possible, however, that these proteins are present in serum but are undetectable because their circulating concentration is much lower than in the microsomes. It should also be emphasized that several major proteins (nos. 21, 24, 25, 27, 35–40, and 43) characteristic of the microsomal released subfraction are not present in the immunoprecipitates. It is therefore unlikely that these proteins represent precursors of serum proteins.

A comparison of the labeling pattern of proteins in the released subfraction and in immunoprecipitates obtained from RM labeled in vivo with [^{3}H]leucine for 5 min (Fig. 12) demonstrated that the radioactivity in serum albumin (band no. 33) representing the major labeled peak was quantitatively transferred to the immunoprecipitate (compare Fig. 12 *a* and *b*). Fig. 12 shows that labeling patterns in supernates (Fig. 12 *c*) were closely complementary to those in immunoprecipitates (Fig. 12 *b*). Although all content proteins precipitated by the rabbit antiserum were labeled within a 5-min interval (Fig. 12 *b*), several proteins which showed no cross-reactivity with serum proteins (nos. 21, 39, 40, and 42), (Fig. 12 *b*), also had high levels of [^{3}H]leucine incorporation.

Released subfractions obtained from short-term labeled RM and SM ([^{14}C]leucine, 30 min) were also analyzed by immunoelectrophoresis on agar-coated slides, using rabbit antirat serum. Three clear precipitation arcs were recognized (Fig. 13

[2] Because of its high mobility the L band is found with the front.

TABLE III

*Immunoprecipitation of Recently Synthesized [^3H]Leucine-Labeled Serum Proteins in Microsomal Subfractions**

		RM‡		SM‡	
	Incubated with	Total dpm × 10^{-4}	Percent precipitable	Total dpm × 10^{-4}	Percent precipitable
Released subfraction (0.15% Triton X-100)	Antiserum	62.37	50	51.57	49
	Antiserum & rat serum	60.52	25	56.68	29
	Control serum	58.10	2	55.56	1
Sedimentable subfraction solubilized in 1% Triton X-100	Antiserum	34.12	14	18.10	14
	Antiserum & rat serum	33.90	23	19.44	16
	Control serum	30.34	9	17.48	7

* For details see Material and Methods.

‡ Microsomal subfractions are those listed in Table I.

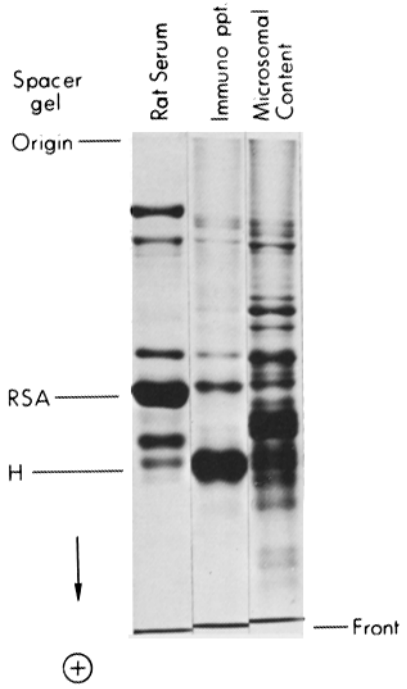

FIGURE 11 Identification of serum proteins in the microsomal content. The content of RM released by 0.15% Triton X-100 was treated with rabbit antiserum against rat serum as described in the text. The total microsomal content, immunoprecipitate, and rat serum (80 μg protein) were processed for electrophoresis and analyzed on 6-mm SDS acrylamide (7%) gels. The amount of immunoprecipitate loaded allows a direct comparison with the microsomal content from which it was obtained.

a), which were shown by autoradiography to correspond to newly synthesized proteins, labeled during the 30-min interval (Fig. 13 *b*).

DISCUSSION

The preceding results demonstrate that in addition to the set of proteins of membrane-bound ribosomes, rat liver microsomes contain more than 50 different species of polypeptide chains which cover a molecular weight range extending from about 10,000 to more than 200,000 Daltons (see also Neville and Glossmann, 1971; Dehlinger and Schimke, 1971). The set of proteins which represents the cisternal content and is selectively released by low detergent concentration is nearly the same as that extracted from microsomes by sonication or mechanical disruption through a French press. This set consists of approximately 25 different polypeptide chains (with a molecular weight ranging from 50,000 to 200,000 Daltons), which represents less than 20% of the total protein, but contains a large proportion (~60%) of the radioactivity incorporated into microsomes after short times of labeling "in vivo" with [^3H]- or [^{14}C]leucine, or with [^3H]glucosamine.

Considerable evidence indicates that the easily released proteins represent the content of microsomal vesicles which escapes through detergent-induced membrane discontinuities. We have previously shown that these proteins are not exposed on the outer face of microsomes. In intact microsomes they were found to be inaccessible to

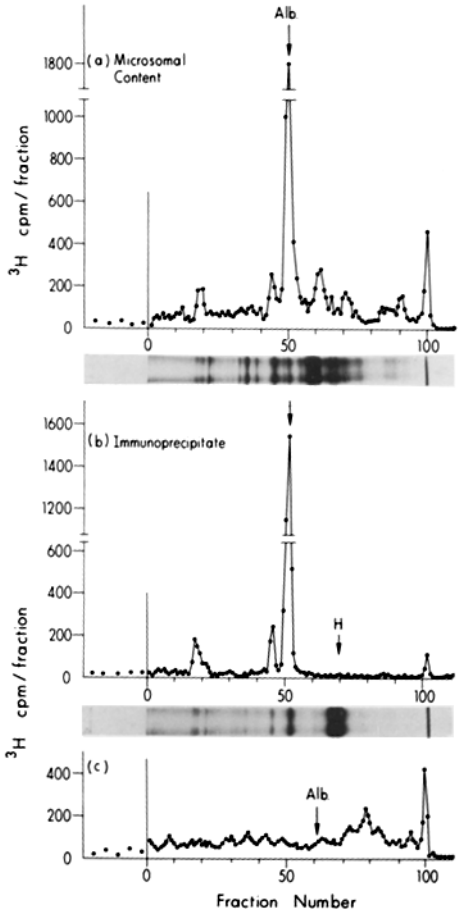

FIGURE 12 Recently synthesized serum proteins isolated from the microsomal content. The content from RM ([^{3}H]leucine, 5 min, see Table I) was treated with antiserum against rat serum (for details see Materials and Methods) to precipitate serum proteins synthesized in RM. Aliquots of microsomal content (*a*), redissolved immunoprecipitate (*b*), and supernate from the immunoprecipitation (*c*) were processed for electrophoresis and analyzed as described in the legend to Fig. 5.

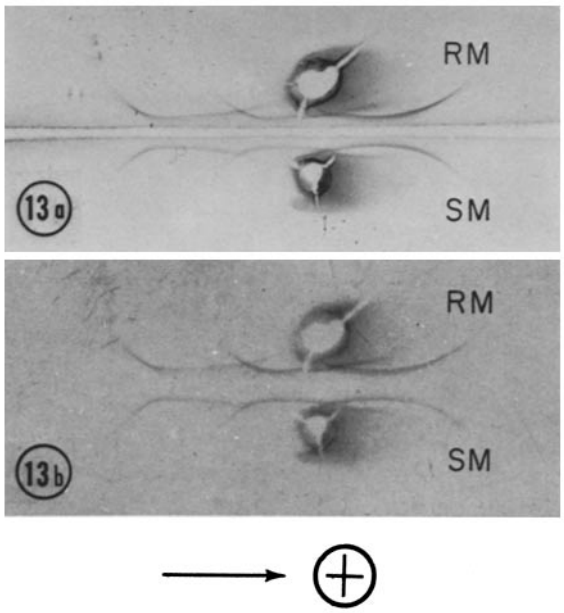

FIGURE 13 Immunoelectrophoresis and autoradiogram of serum proteins in the microsomal content. 5 μl of the eightfold concentrated supernates released by 0.15% Triton X-100 from RM (~670 dpm) and SM (~480 dpm) ([^{14}C]leucine, 30 min) were applied in the two central holes of the agar-coated slide. After electrophoresis, the antibody reservoir was filled with 20 μl of rabbit antiserum against rat serum. After 24 h (4°C), the slides were washed in saline (48 h) and stained with amido black (*a*). For autoradiography (*b*), the dried slide was exposed with X-ray film for 14 days.

added proteases (Kreibich et al., 1973), or to an exogenous lactoperoxidase-iodinating system (Kreibich et al., 1974), unless low detergent concentrations were added which produced reversible microsomal openings.

Our observations demonstrate that several proteins in the content subfraction are secretory products, most probably manufactured in the ER and discharged into the cisternal cavity for eventual release into the bloodstream. Electrophoretic and immunological characterization and the patterns of amino acid and glucosamine incorporation show that several serum proteins of hepatic origin are represented in the released subfraction. Polypeptide bands nos. 10–13, 16, 30, and 33 either corresponded in mobility and carbohydrate distribution to polypeptides found in serum, or cross-reacted immunologically with an antiserum against total rat serum. In particular, some of these bands (nos. 10–13, and 33) contained a large fraction of the radioactivity incorporated into rapidly synthesized proteins (highly labeled after 2, 5, or 30 min). The weak Coomassie blue band no. 33, which contained no carbohydrate, coincided with the highest peak of [^{3}H]leucine radioactivity incorporated during short periods of in vivo labeling. The same band contained only a negligible portion of the microsomal radioactivity released after a long-term labeling procedure which ended 1 day before sacrifice. These two facts show that band no. 33

corresponds to a microsomal protein rapidly synthesized and transported out of the ER. This protein was shown by immunological methods to be serum albumin. Recently the existence of an intracellular precursor of rat serum albumin has been reported. This precursor, identified on the basis of a different isoelectric point (Russel and Gellert, 1973), had the same mobility as serum albumin in SDS acrylamide gel electrophoresis and was immunologically indistinguishable from circulating serum albumin.

The labeling patterns and the immunological properties of the constituents in microsomal subfractions show that the fate and metabolic stability of proteins in the microsomal content is very complex. This is best illustrated by the labeling behavior of those polypeptides in the released subset which were not precipitated with antibodies against rat serum. Some of these were labeled with [^{3}H]leucine after 2 and 30 min (bands nos. 21, 39, 40, and 42) and hence were relatively rapidly synthesized. Others, however, were only negligibly labeled after 30 min and acquired higher levels of labeling only after the long-term treatment, and in this respect were similar to proteins (such as cytochrome P 450) which are constituents of microsomal membranes. Rapidly synthesized polypeptides which did not cross-react with antirat serum could possibly be precursors of serum proteins which have not yet acquired antigenic determinants. It appears more likely, however, that such content proteins represent products of the ER which are first discharged into the cisternae but are retained intracellularly by subsequent diversion into other membrane-bound compartments. In this respect it is interesting to note that an ER origin has been suggested for lysosomal (de Duve and Wattiaux, 1966; Goldstone and Koenig, 1972) and peroxisomal proteins, and evidence has been presented to suggest that recently synthesized catalase is contained in rough microsomes (Higashi and Peters, 1963 *a,b;* Kashiwagi et al., 1971; but see Redman et al., 1972; and Lazarow and de Duve, 1973). In the gel system used by us, catalase has the same mobility as band no. 35, which is also found in the released subfraction. However, this may be coincidental since catalase is not a glycoprotein and band 35 shows some [^{3}H]glucosamine incorporation.

Since smooth microsomes and Golgi elements present in the same fraction are known to contain enzymes such as glycosyl transferases which modify secretory proteins (Fleischer and Fleischer, 1970; Ehrenreich et al., 1973; Bergeron et al., 1973), we expected to detect differences in the electrophoretic patterns of content proteins from rough and smooth microsomes. These were not observed. Furthermore the mobility of individual labeled peaks in subfractions of both types of microsomes did not change significantly with the labeling interval. This may only indicate that limited growth of glycosidic chains was not detectable by gel electrophoresis.

Two main possibilities should be mentioned in considering the origin of the metabolically stable (nonlabeled in short times) but releasable microsomal proteins (such as proteins 24, 25, and 27). One is that they represent stable membrane components loosely attached to the inner face of the microsomes, as proposed for membrane-associated proteins (Fleischer et al., 1971) or peripheral membrane proteins (Singer, 1971). Such localization has been suggested for nucleoside diphosphatase and other enzymes which are released from microsomes by mild detergent or mechanical treatment (Kuriyama, 1972). Another possibility is that the releasable but metabolically stable proteins are residents of the ER lumen which may exert a modifying function on other content proteins, or maintain the quality of the cisternal environment, but are not destined for secretion and are prevented from transfer to other compartments by mechanisms as yet unknown.

It is interesting to note that with the major exception of serum albumin (band no. 33) and in accordance with the fact that many serum proteins are glycoproteins, most proteins in the released subfraction were stained with the PAS method and showed at least some incorporation of [^{3}H]glucosamine within a 30-min interval. Not only were most released proteins glycoproteins, but almost all microsomal glycoproteins were at least partially released by the low detergent concentration procedure or by mechanical disruption. In fact, only one microsomal glycoprotein (no. 49) was completely absent from supernates and was visible in sediments as a PAS-stained band. Other glycoproteins of high molecular weight (bands nos. 1, 2, 3, >200,000 Daltons mol wt), were not extensively released, but they were recognized as containing carbohydrate mainly because they were highly labeled with [^{3}H]glucosamine in a 30-min interval. It is interesting that these large molecular weight proteins showed very low levels of [^{3}H]leucine incorporation within the same time interval. Barring the unlikely possibility that these are

proteins extremely deficient in leucine, their labeling kinetics suggests that they are relatively stable membrane constituents. It is possible therefore that the [^{3}H]glucosamine incorporation of these proteins reflects an independent carbohydrate turnover such as may be expected of membrane-bound glycosyl transferases. For some glycoproteins the levels of [^{3}H]glucosamine corresponded to a high rate of protein synthesis (bands nos. 10–13, 39, 42).

A comparison of electrophoretic and labeling patterns of subfractions from rough and smooth microsomes obtained after labeling with [^{3}H]leucine for 2 and 30 min, and with [^{3}H]glucosamine for 30 min, showed qualitatively similar protein compositions and labeling behavior in both microsome types. Smooth microsomes were found to have a much higher albumin content than RM, as was previously reported by other authors (Glaumann, 1970; Peters et al., 1971). However, the specific activity of albumin after short times of labeling with leucine (2 or 30 min) was much lower than in RM, which could be expected from a transfer of content from rough to smooth endoplasmic reticulum during the secretory cycle. Smooth microsomes labeled for short periods of time showed a 10-fold higher incorporation of [^{3}H]glucosamine than RM. This observation is also consistent with the sequence of glycosidation steps which is known to involve enzymes in rough and smooth endoplasmic reticulum membranes after synthesis, and release of recently synthesized proteins from membrane-bound ribosomes (Molnar et al., 1965; Schachter et al., 1970; Redman and Cherian, 1972).

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