Evidence for Rapid Turnover of Hepatic Endoplasmic Reticulum and Its Possible Relationship to Secretion*

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(Received for publication, February 11, 1980, and in revised form, October 13, 1980)

Rat liver microsomal membranes were purified in order to remove membrane-associated secretory products. Measurements of the decay of the newly synthesized protein of these membranes in vivo were carried out at short time intervals after the protein was labeled by the administration of radioactive leucine. The result of these measurements suggest that the membranes are synthesized and degraded at approximately the same rapid rate as the synthesis and secretion of membrane-associated secretory products. Evidence that the highly dynamic protein of the purified membranes is indeed membrane protein is provided by the observations indicating: that this protein is immunochemically distinct from serum proteins, which are the major secretory product of liver; that many different protein components of the membranes turn over at similarly rapid rates; and that the biosynthesis of these proteins is specifically stimulated by the administration of phenobarbital, which is known to stimulate biosynthesis of hepatic endoplasmic reticulum. These findings suggest that in liver, as had been proposed earlier for the myeloma cell, unidirectional membrane flow, accompanied by rapid synthesis at the origin of flow and rapid degradation of the membranes at or near the terminus of flow, may be the mechanism for the intracellular transport of secretory product.

Evidence recently presented from this laboratory suggests that intracellular membranes of actively secreting myeloma cells are synthesized and degraded at approximately the same rate as the synthesis and secretion of immunoglobulin light chain, which is the predominant secretory product of these cells. From this and other evidence, it was proposed that unidirectional membrane flow accompanied by rapid synthesis of the membrane at the origin of flow and its rapid degradation at or near the terminus of flow may be the mechanism for intracellular transport of secretory product in these cells (1, 2). The purpose of the present investigation is to determine whether a similarly rapid turnover of intracellular membranes and, therefore, presumably a similar mechanism for the intracellular transport of secretory product occurs in liver.

The turnover of hepatic intracellular membranes has been the subject of numerous investigations (3–7). However, in order to prevent turnover of the membranes from being obscured by the intracellular turnover of membrane-associated secretory products, most of these earlier studies did not start measuring turnover, as indicated by the decay of pulse-labeled membrane components, until after most of the pulse-labeled secretory product had been secreted by the cell. Because of this delay, these studies would not have detected the presence of highly dynamic membrane components.

The present study was made possible by the development of methods for separating the membranes from membrane-associated secretory products (8, 9). Utilizing one of these methods (8), we have examined the decay of membrane protein at short time intervals after the administration of radioactive amino acids and have obtained evidence suggesting that over one-half of the newly synthesized protein of the membranes turns over with a half-life of approximately 1 h. Evidence that this highly dynamic protein is indeed membrane protein is suggested by data indicating (a) that this protein is immunochemically distinct from serum proteins, which are the major secretory products of liver; (b) that many different protein components of the membrane fractions undergo a similarly rapid turnover; and (c) that the biosynthesis of these dynamic proteins is stimulated by phenobarbital administration, which is known to stimulate specifically the biosynthesis of endoplasmic reticulum (10, 11). Finally, kinetic evidence suggests that the dynamic membrane protein turns over at approximately the same rate as the intracellular turnover of membrane-associated secretory products. These observations suggest that the proposed mechanism for intracellular transport of immunoglobulins by the myeloma cell also may apply to the hepatic secretory process.

MATERIALS AND METHODS

Measurement of Protein Turnover in Vivo—After adult male Carworth CFE rats weighing 150 to 250 g had been fasted overnight, 10 μCi of [1-14C]leucine (25 mCi/mmol, New England Nuclear Inc.) were administered by tail vein injection. The rats were then sacrificed at specified time intervals. The livers were quickly excised, chilled in ice-cold 0.25 M sucrose for 5 min and then homogenized in 10 ml of 0.25 M sucrose/g (wet weight) of liver with a Willena Polytron homogenizer, model PT-10, operated for 7 s at maximum speed. This and all subsequent operations were carried out at 0–4°C.

Cell Fractionation and Purification of Microsomal Membranes—The liver homogenates were centrifuged at 12,000 rpm for 20 min in a Sorvall SS-34 rotor to remove cell debris, nuclei, and mitochondria. Microsomes were sedimented from the supernatant layer by centrifugation at 30,000 rpm for 30 min in a Spincso 30 rotor. The resulting supernatant layer is designated as "cytosol."

Microsomal membranes were purified by a modification of the multiple salt extraction procedure of Weihing et al. (8). In the present study, microsomes from 2.5 g of liver were suspended by homogenization in 10 ml of 0.14 M sodium chloride. The suspension was diluted to 25 ml with 0.14 M sodium chloride, mixed, and then centrifuged as described for sedimenting microsomes. The pellet was washed in a similar manner with 25 ml of 1.0 M sodium chloride and finally with 25 ml of a solution containing 0.1 M sodium carbonate and 0.1 M sodium bicarbonate. The final washed pellet is designated as "microsomal membranes" or "membranes."

* This research was supported by the University of Connecticut Research Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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These membrane preparations still contain about 10% of the immunochromically detectable serum protein originally associated with the membranes (8). This protein was removed by extracting the membranes with dilute deoxycholate according to the following procedure. Membranes from 1.25 g of liver were suspended by homogenization in 2 ml of 0.25 M sucrose, and 0.15 ml of 3% sodium deoxycholate, which had been adjusted to a pH of 7.6, was slowly added while stirring. The suspension, after dilution to 6 ml with 0.25 M sucrose (which gives a final deoxycholate concentration of 0.075%), was centrifuged at 40,000 rpm for 90 min in a Spinco 40 rotor. The resulting supernatant layer, which was carefully decanted, is designated as the "deoxycholate extract." The pellet, which has been shown to contain tissue of open vesicles (8), is designated as "deoxycholate-extracted membranes." It is important to note that this step, in contrast to the preceding salt extraction steps, is accompanied by significant disruption of the membranes as indicated by the release of significant amounts of phospholipid and of the activities of several microsomal enzymes thought to be integral membrane components (8).

Fractionation of Microsomal Membrane Proteins by SDS-Acrylamide Gel Electrophoresis—Preliminary deoxycholate-extracted microsomal membranes (from 1.25 g of liver) were suspended by homogenization in 1 ml of distilled water. Aliquots of this suspension were used for electrophoresis as well as for determining the levels of labeled protein described in the next section. The deoxycholate extracts of the microsomal membranes were concentrated approximately 10-fold by ultrafiltration in a model 12 Amicon filter apparatus using a PM-10 Amicon filtering membrane. 0.25-ml aliquots of suspensions of deoxycholate-extracted membranes and concentrated deoxycholate extract were solubilized, submitted to electrophoresis, and stained with Commassie blue exactly as described previously (1). Because the patterns of stained protein from deoxycholate extracts tended to be somewhat diffuse, the solubilized extracts were usually mixed with an equal volume of solubilized deoxycholate-extracted membranes (unlabeled) before being submitted to electrophoresis. This carrier was added in order to provide clearly discernible electrophoretic bands of stained protein, which served as visual reference points for determining the distribution of newly synthesized membrane proteins.

Isolation and Assay of Radioactive Protein—Radioactive protein in microsomal membranes and membrane subfractions obtained by extraction with deoxycholate were prepared for counting essentially as described by Mans and Novelli (12). Labeled cell fractions (50 to 150 μl) were applied to a disc of Whatman No. 3 chromatography paper, 2.3 cm in diameter, and dried by exposure to a stream of warm air for 15 s. Batches of the discs were washed successively with ice-cold 10% trichloroacetic acid and 0.1 M unlabeled L-leucine, cold 5% trichloroacetic acid, 5% trichloroacetic acid at 90°C, ethanol/ether (1/1), twice with ethyl ether, and finally dried in air. Each disc was placed in a standard 20-ml glass scintillation vial and 5 ml of scintillation mixture (0.4% 2,5-diphenyloxazole and 1,4-bis-2-[5-phenyloxazolyl] benzene in toluene) was added. The samples were counted in a Packard Tri-Carb liquid scintillation spectrophotometer. Background was determined on filter paper disc carried through the washing procedure with the sample discs. In general, no differential quenching among samples was observed.

The distribution of labeled protein on SDS-acrylamide gels was determined by mechanical (Yeda mechanical slicer) or free-hand slicing of the gels into sections less than 2 mm thick. The slices were processed for the determination of radioactivity as described previously (1).

Preparation of Serum Protein—Freshly collected rat blood was allowed to clot for 2 h at room temperature. After standing overnight at 0-4°C, the serum was carefully removed with a pipette and centrifuged at 3000 rpm for 30 min in a Sorvall rotor in order to remove any remaining red blood cells. Radioactive serum protein was prepared in a similar manner from blood obtained from a 150-g Carworth CPE rat 45 min after it had received an intraperitoneal injection of 100 μCi of L-[^14]C]leucine. Immunoprecipitation of Rat Serum Protein—Serum protein was precipitated with antisem as described by Peters (13) and the precipitate was washed essentially as described by Redman (14). 0.1 ml of rabbit antisem to rat serum (purchased from Hyland Laboratories and centrifuged immediately prior to use) was added to a series of tubes containing increasing amounts of serum protein in 0.5 ml of saline solution. The tubes were swirled gently and then incubated at 37°C for 1 h and then at 0-4°C for at least 17 h. All further steps were carried out at 0-4°C. The tubes were centrifuged in an International clinical centrifuge at top speed for 10 min. The resulting supernatant layer is designated as "immunosupernatant." The precipitate, which was washed 5 times by suspension in 0.5 ml of 0.9% sodium chloride followed by centrifugation, is designated as the "immunoprecipitate." In experiments in which immunoprecipitation of radioactive proteins was studied, the radioactivity of the protein in the immunoosupernatant fraction and the immunoprecipitate was assayed by the method described above. For the determination of specific radioactivities, aliquots of these fractions (immunoprecipitates were suspended by homogenization in a minimum volume of 0.9% sodium chloride) were also assayed for protein as described below.

Phenobarbital Treatment—Sodium phenobarbital, at a dose of 100 mg/kg body weight, was administrated by intraperitoneal injection of a solution containing 50 mg of sodium phenobarbital/ml of 0.9% sodium chloride.

Protein Analysis—Protein was assayed by the method of Lowry et al. (15). Crystalline bovine serum albumin was used as the standard.

RESULTS

Turnover of Microsomal Protein—A typical experiment showing the distribution of labeled protein in microsomal membranes and the cytosol isolated from rats at various time intervals after they had received a single injection of L-[^14]C]leucine is illustrated in Fig. 1. At short time intervals after injection, the membranes exhibit a very high initial specific activity that declines rapidly and then, with less than one-half of the radioactivity remaining, much more slowly. The biphasic nature of the decay suggests the presence of 2 kinetically distinct classes of newly synthesized protein; one that decays with a half-life of approximately 1 h and the second, which is much more stable. The latter probably corresponds to the newly synthesized microsomal membrane protein that other workers had observed at much longer time periods.

FIG. 1. The in vivo decay of radioactive protein in microsomal membranes and cytosol of rat liver. The experiments were performed as described under Materials and Methods. The specific radioactivities are plotted as a function of time after the injection of [^14]C]leucine.

Note: The abbreviation used is: SDS, sodium dodecyl sulfate.
intervals after the injection of labeled amino acids (3-7). Fig. 1 also shows that the much lower specific activity of the cytosolic protein remains relatively unchanged over the time course of the experiment.

Because the membranes still contain about 10% of the serum protein originally associated with the microsomes (8), this experiment does not rule out the possibility that the dynamic protein represents newly synthesized, membrane-associated serum protein, which is known to turn over rapidly.

![Graph showing decay of radioactive protein](image)

**Fig. 2.** The decay of radioactive protein in deoxycholate-extracted microsomal membranes and the deoxycholate extract. The experiments, including the extraction of microsomal membranes with 0.075% deoxycholate, were performed as described under "Materials and Methods." The points and brackets refer to the average and standard deviation for measurements carried out on 3 rats/time point.

![Graph showing precipitates](image)

**Fig. 3.** Precipitation of radioactive rat serum protein by rabbit antiserum does not rule out the possibility that the dynamic protein represents newly synthesized, membrane-associated serum protein, which is known to turn over rapidly.

![Graph showing recoveries](image)

**Fig. 4.** Immunoprecipitation of radioactive protein in the deoxycholate extract of microsomal membranes by rabbit antiserum to rat serum protein. Carrier rat serum protein (8.5 µg) was added to increasing amounts of the extract which was diluted to 0.5 ml with 0.9% sodium chloride before the addition of 0.1 ml of rabbit antiserum. The amount of protein and radioactivity in the washed immunoprecipitates was determined as described under "Materials and Methods." The microsomal membranes were isolated from a 160-g rat injected 1 h previously with 10 μCi of [14C]leucine. The point (A) on the ordinate refers to the amount of immunoprecipitate obtained with the carrier serum protein alone.

**Table 1.**

<table>
<thead>
<tr>
<th>µg of protein</th>
<th>Recoveries of total radioactive protein</th>
<th>Recoveries of total radioactive protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>96</td>
<td>39%</td>
<td>23%</td>
</tr>
<tr>
<td>192</td>
<td>41%</td>
<td>20%</td>
</tr>
<tr>
<td>268</td>
<td>46%</td>
<td>16%</td>
</tr>
<tr>
<td>364</td>
<td>46%</td>
<td>16%</td>
</tr>
<tr>
<td>490</td>
<td>50%</td>
<td>17%</td>
</tr>
</tbody>
</table>

Experiments were, therefore, carried out to examine the distribution of the dynamic protein after more complete removal of serum protein from the membranes by extraction with 0.075% sodium deoxycholate (8). The results shown in Fig. 2, and also to be shown in Fig. 8, indicate that although a significant proportion of the protein undergoing rapid turnover remains associated with the detergent-extracted membranes, a much larger proportion is released from the membranes by the detergent. In making this comparison, it should be noted that 40-43% of the total microsomal membrane protein is solubilized by this extraction procedure. The tendency of the highly dynamic protein to be selectively extracted by this detergent would seem to suggest that it represents membrane-associated secretory products. However, because the membranes are partially disrupted by this treatment (8), the possibility still remains that much of this protein represents newly synthesized membrane protein which, for some reason, is less stable to detergent extraction than previously synthesized membrane protein.

**Immunochromical Identification of Rat Serum Protein**—In an attempt to resolve the above question, the following experiments were carried out to determine by immunochromical means whether the protein undergoing rapid turnover in the deoxycholate extract corresponds to rat serum protein. Preliminary experiments to determine optimal conditions for immunoprecipitation of rat serum protein in the presence of 0.075% sodium deoxycholate are shown in Fig. 3. The titration of increasing amounts of radioactive rat serum protein against antiserum to the protein indicates that in the range between...
8 and 80 μg of added serum protein, a linear relationship exists between the amount added and the amount of immunoprecipitate recovered. It is important to note that throughout this range 90% of the radioactive serum protein is removed from the dilute deoxycholate solution by immunoprecipitation. On the other hand, recoveries of the serum protein in the immunoprecipitate are considerably lower with maximal recoveries of approximately 70% occurring between 40 and 80 μg of added serum protein. The difference between these values undoubtedly represents limited stability of the immunoprecipitate during the washing procedure.

A titration curve for the immunoprecipitation of newly synthesized protein from the deoxycholate extract of microsomal membranes is shown in Fig. 4. In this experiment, the deoxycholate extract was isolated from rats at a time (1 h after [14C]leucine injection) when the highly dynamic protein of the extract is extensively labeled (see Fig. 2). Also, each immunoprecipitation was carried out in the presence of 8.5 μg of carrier rat serum protein, which is the minimum quantity required for efficient removal of serum protein from solution by immunoprecipitation (see Fig. 3). The data in Fig. 4 indicate a linear relationship between the amount of added extract and the amount of protein and radioactivity recovered in the immunoprecipitate.

In Table I, it is shown that at all but the lowest concentrations of the deoxycholate extract, between 45 and 50% of the total radioactivity resists precipitation by the antibody. Since 90% of serum protein is precipitated under these conditions (Fig. 3), this result suggests that nearly one-half of the radioactive protein in the extract is immunochemically distinct from serum protein.

Evidence that an even larger fraction of this protein is immunochemically distinct from serum protein is provided by the data in Table I showing that only 16 to 23% of the radioactive protein in the deoxycholate extract is recovered in the immunoprecipitates, which were washed in order to remove nonspecifically bound protein. When corrected for the 60 to 70% recovery of authentic rat serum protein in immunoprecipitates prepared under the same conditions, these data suggest that only about one-third of the radioactive protein present in the deoxycholate extract 1 h after [14C]leucine injection appears to correspond immunochemically to rat serum protein.

The levels of radioactive protein in the immunosupernatant fraction and the immunoprecipitate from deoxycholate extracts isolated from rats at time intervals ranging from ½ h to 17 h after the injection of [14C]leucine are shown in Fig. 5. It can be seen that the newly synthesized protein in the immunosupernatant fraction undergoes approximately the same biphasic decay as that observed for the membranes from which the fraction was derived. It can also be seen that its initial rate of decay is approximately the same as that observed for the essentially complete decay of newly synthesized serum protein in the immunoprecipitate.

**Effect of Phenobarbital upon the Biosynthesis and Decay of Microsomal Membrane Proteins**—Since phenobarbital is known to stimulate the biosynthesis of hepatic endoplasmic reticulum (5, 6, 10, 11), it was of particular interest to determine whether the newly synthesized protein in the deoxycho-

![Fig. 6](image_url)
extracts were subjected to indicated times after \(['%]\)leucine injection. Extracts of microsomal membranes isolated from radioactive serum and nonserum protein in the deoxycholate 5. The points and brackets were treated with phenobarbital 6 h before the administration be close to the minimum required for phenobarbital stimula-
tion of \([''C]\)leucine. This time interval, which has been found to expected if it were a constituent of these membranes. Rats late extract responds to the drug in a manner that would be expected if it were a constituent of these membranes. Rats were treated with phenobarbital 6 h before the administration of \([''C]\)leucine. This time interval, which has been found to be close to the minimum required for phenobarbital stimulation of amino acid incorporation into the microsomal membranes (5), was used to avoid any secondary, less specific effects of the drug. The experiments shown in Fig. 6 indicate that this treatment results in a significant increase in the amount of radioactivity appearing in the highly dynamic protein of the deoxycholate extract but does not significantly alter its rate of decay. The experiments shown in Fig. 7 indicate that phenobarbital specifically stimulates incorporation of \([''C]\)leucine into that portion of the deoxycholate-extractable protein that is antigenically distinct from serum protein. The lack of any effect of the drug upon the level of radioactivity incorporated into cytosolic protein (Fig. 8) provides a further indication of the specificity of this response to the drug.

Comparison of Relative Rates of Turnover of Different Microsomal Membrane Proteins Separated by SDS Acryl amide Gel Electrophoresis—In a series of experiments very similar to those reported by Kreibich and Sabatini (17), deoxycholate-extracted membranes and deoxycholate extracts isolated from rats injected with \([''C]\)leucine were submitted to SDS-acrylamide gel electrophoresis and the distribution of radioactive protein in a series of thin sections from various regions of the gels was measured. As noted by these earlier workers (17), approximately one-half of the newly synthesized protein of these fractions was observed to migrate as distinct peaks whose positions in the gels coincide with major bands of stained protein. A typical pattern of stained protein for deoxycholate-extracted membranes and the relative positions of the radioactive peaks from extracted membranes and extracts are shown in Fig. 9. The apparent molecular weights of the peaks are shown in Table II and the distribution of radioactivity in regions of the gel containing the peaks is shown in Tables III and IV. Again, as found by Kreibich and Sabatini (17), most of the peaks from deoxycholate extracts coincided very closely, in terms of their positions on gels, with major peaks from deoxycholate-extracted membranes. The only exception is a peak that seems to be present only in the extract (E3 in Fig. 9 and Table II). As suggested by its apparent molecular weight of 67,000, this peak probably represents serum albumin.

The distribution of newly synthesized protein among the electrophoretic bands of deoxycholate-extracted membranes and the extracts isolated from rats at increasing time intervals after the administration of \([''C]\)leucine is also shown in Tables III and IV. It can be seen that despite the decay of approximately 27% in the level of labeled protein in the extracted
membranes of normal and phenobarbital-treated rats (Fig. 6) and the decay of approximately 80% in the extracts (Fig. 8), the distribution of labeled protein among the various bands from these two fractions shows little consistent change during the decay period. This comparison suggests that different protein components of the membranes turn over at similarly rapid rates. One possible exception is an apparently more rapid initial decay of newly synthesized protein recovered in band E3 corresponding to rat serum albumin (see Table IV). However, this band represents only a small, somewhat variable fraction of the serum protein originally associated with the microsomes (8). Furthermore, because this band does not co-migrate with a distinct band of stained protein (see Fig. 9), its isolation from the gel may be subject to larger errors than the isolation of other bands of newly synthesized protein. Therefore, it is not clear whether the observed differences in the distribution of newly synthesized protein in this band actually represent a difference in its rate of decay. Finally, recoveries of newly synthesized protein from regions of the gel where the protein does not migrate as distinct bands, as shown by the last row of Tables III and IV, appear to remain relatively unchanged during the decay period. This observation suggests that in general these proteins decay at the same rate as the proteins migrating as distinct bands.

As indicated by the close similarity between the distributions of newly synthesized proteins in untreated and phenobarbital-treated rats, the drug appears to stimulate leucine incorporation to an equal extent in all protein fractions that can be resolved from the membranes by SDS-acrylamide gel electrophoresis. Furthermore, as indicated by the relative constancy of distribution of labeled proteins in the gels

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**Table II**

<table>
<thead>
<tr>
<th>Band no.</th>
<th>Deoxycholate-extracted membranes</th>
<th>Deoxycholate extract</th>
<th>Apparent molecular weight $\times 10^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>-180</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td>140-130</td>
<td>105-100</td>
<td></td>
</tr>
<tr>
<td>R3</td>
<td>E1</td>
<td>E2</td>
<td>E3</td>
</tr>
<tr>
<td>R4</td>
<td>86-83</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>R5</td>
<td>62</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>R6</td>
<td>E4</td>
<td>54-49</td>
<td></td>
</tr>
<tr>
<td>R7</td>
<td>E5</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>R8</td>
<td>35</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>R9</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

**Table III**

The distribution of radioactive protein in electrophoretic bands of deoxycholate-extracted membranes at various times after administration of $[^{14}C]$leucine to normal and phenobarbital-treated rats

The extracted membranes were obtained from the experiments shown in Fig. 2 (untreated rats) and Fig. 8 (treated rats). The membranes, which were combined from 3 rats for each time point, were solubilized and submitted to SDS-acrylamide gel electrophoresis as described under "Materials and Methods." Co-migrating bands of stained and radioactive protein (see Fig. 9) were isolated for the determination of radioactivity by free hand slicing of the gels into 1 or more segments containing each of the bands. These segments, which were sliced to be less than 2-mm thick, as well as other regions of the gel, were assayed for radioactivity as described previously (1). Approximately 90% of the applied radioactivity was recovered from the gels.

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**Fig. 9.** SDS-acrylamide gel electrophoresis of deoxycholate-extracted membranes and deoxycholate extracts prepared from rats injected with $[^{14}C]$leucine 1 h before sacrifice. Electrophoresis and staining of the gels were carried out as described under "Materials and Methods" and under "Results." The distribution of radioactive protein was determined by slicing regions of gels into a series of 1-mm sections with a Yeda mechanical slicer (using stained protein bands as visual reference points) and assaying the slices for radioactivity as described earlier (1). The photograph shows a stained gel of deoxycholate-extracted membranes, which was also added as a carrier during electrophoresis of deoxycholate extracts (see "Materials and Methods"). The brackets refer to regions of gels containing distinct peaks of radioactive protein.
The distribution of radioactive protein in electrophoretic bands of deoxycholate extracts of microsomal membranes at various times after administration of \(^{[14C]}\)leucine to normal and phenobarbital-treated rats.

The extracts, combined from 3 rats for each time point, were obtained from the experiments described in Fig. 2 (untreated rats) and Fig. 6 (phenobarbital-treated rats). The extracts were mixed with carrier deoxycholate extracted membranes and then solubilized and submitted to electrophoresis as described under "Materials and Methods." The designated bands of radioactive protein and other regions of the gel were assayed for radioactivity as described in Table III. Approximately 90% of the applied radioactivity was recovered from the gels.

<table>
<thead>
<tr>
<th>Band No.</th>
<th>Normal rats</th>
<th>Phenobarbital-treated rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time after (^{[14C]})leucine injection</td>
<td>% radioactivity (^a)</td>
</tr>
<tr>
<td>E1</td>
<td>(\frac{1}{2}) h</td>
<td>4.5 5.2 6.4 5.8</td>
</tr>
<tr>
<td>E2</td>
<td>1 h</td>
<td>10.0 9.8 10.1 9.2</td>
</tr>
<tr>
<td>E3</td>
<td>2 h</td>
<td>14.8 9.7 9.3 8.4</td>
</tr>
<tr>
<td>E4</td>
<td>(\frac{1}{2}) h</td>
<td>14.4 10.6 14.3 14.6</td>
</tr>
<tr>
<td>E5</td>
<td>1 h</td>
<td>10.8 9.9 11.9 9.0</td>
</tr>
<tr>
<td>Total</td>
<td>(\frac{1}{2}) h</td>
<td>54.5 45.2 51.8 49.2</td>
</tr>
</tbody>
</table>

\(^a\) The values represent the per cent of total radioactivity recovered from the gels.

Fig. 10. SDS-acrylamide gel electrophoresis of newly synthesized protein in immunoprecipitates from a deoxycholate extract of microsomal membranes. The immunoprecipitate, which was isolated as described in Fig. 5 from a rat sacrificed 1 h after it had received an injection of \(^{[14C]}\)leucine, was solubilized, submitted to electrophoresis, and the gel was then assayed for radioactivity as described previously (1). The numbered brackets refer to the positions expected for specific bands of newly synthesized protein in the original deoxycholate extract (see Fig. 9). The positions of human serum albumin (stable dimer and monomer) and cytochrome c, after electrophoresis on a duplicate gel, are indicated by A, B, and C, respectively.

DISCUSSION

Three lines of evidence suggest that the rapid in vivo turnover of protein in hepatic microsomal membranes does not simply represent intracellular turnover of membrane-associated serum proteins. The first is our data indicating that much of this protein is immunologically distinct from serum protein. These data alone do not exclude the possibility that the protein is serum protein which, because of incomplete synthesis, incomplete post-translational modification, or because of its association with the membranes, does not exhibit its final antigenic characteristics. However, detailed kinetic analysis of the biosynthesis and intracellular transport of specific serum proteins seems to rule out the existence (over time intervals longer than a few minutes) of kinetically significant amounts of immunologically reactive form of these proteins or their precursors (18). A second line of evidence is the observation that approximately one-third of the highly dynamic protein remains associated with the membranes after removal of essentially all of the membrane-associated serum protein by extraction with sodium deoxycholate. The third line of evidence is that stimulation of membrane biosynthesis by phenobarbital administration stimulates incorporation of amino acid into this protein but not into membrane-associated serum protein.

The close physical association of a significant portion of the dynamic protein with the membranes and the nature and specificity of its response to phenobarbital administration suggest that its turnover represents rapid turnover of at least part of the membrane itself.

Evidence bearing on this conclusion is provided by SDS-acrylamide gel electrophoresis of the membranes. Our finding that protein components undergoing rapid turnover were distributed in several molecular weight regions of the gel permits us to conclude that the turnover is not due solely to one or two major proteins. Furthermore, our observation that this distribution of protein remains essentially unchanged after its biosynthesis has been stimulated by phenobarbital administration suggests that this response also is not limited to only
a few proteins. Unfortunately, the resolution of these gels does not permit us any conclusions as to precisely how many proteins there are, their molecular weights, or whether they correspond to any bands that can be visualized. Further work is in progress to answer these questions.

The tendency of the highly dynamic protein to be preferentially released from the membranes by 0.075% deoxycholate suggests either that this protein is an unstable precursor of other membrane proteins or that it is derived from a membrane that itself is physically as well as kinetically unstable. The former possibility is raised by evidence from other laboratories. Franke et al. (19) observed a similarly rapid turnover of newly synthesized protein in various intracellular membranes of rat liver and presented evidence suggesting that the turnover represents the flow of the newly synthesized membrane from endoplasmic reticulum to the Golgi apparatus and finally to the plasma membrane where it tends to accumulate (see also Ref. 20). Doyle et al. (21) observed a biphasic decay of fucose-labeled glycoprotein in intracellular membranes of hepatoma tissue culture cells and presented evidence in vitamin A confirmation that the rapid phase of decay represents the transfer of the newly synthesized glycoprotein from an internal membrane pool to the plasma membrane. Finally Craft et al. (22) observed that newly synthesized rat liver cytochrome P-450, normally an intrinsic membrane component, exists in a form that tends to be released from microsomal membranes to a much larger extent than mature cytochrome P-450. These authors tentatively proposed that a population of the cytochrome which is translocated across the membranes of rough endoplasmic reticulum is transferred via the cisternae of the endoplasmic reticulum to the Golgi apparatus. However, it seems unlikely that the dynamic membrane component observed by us is merely a precursor of other hepatic cell membranes. This conclusion is suggested by the fact that the microsomal membranes examined in this study were prepared from vigorously homogenized cells (8) and, therefore, would be expected to contain fragments derived from many if not most of the membranous elements of the hepatic cell, except for mitochondria which are known to turn over slowly (23).

The fact that microsomes contain membranes derived from several different organelles raised the possibility that the dynamic protein is localized in a minor, unstable cell organelle. However, an earlier study (24) has shown that during both sustained amino acid incorporation and pulse-chase experiments in liver slices, the newly synthesized protein is distributed fairly uniformly in the membranes of isolated rough and smooth microsomes. This distribution parallels the distribution of endoplasmic reticulum, but not of other cell membranes (e.g. Golgi and plasma membranes) which are recovered predominately in the smooth microsomal fraction.

The available data, therefore, suggest that the dynamic and stable components of newly synthesized protein represent two kinetically distinct classes of endoplasmic reticulum: one that turns over with a half-life of approximately an hour and the second more stable component which has been shown by others (3-7) to turn over with a half-life of 2 to 3 days. This conclusion is strongly supported by evidence indicating that both NADPH-cytochrome P-450 reductase (25) and the heme of cytochrome P-450 (26-28) undergo a biphasic decay that is very similar to that reported here for total microsomal membrane protein. In liver both of these functional proteins are membrane constituents that are confined largely, although apparently not exclusively, to the endoplasmic reticulum (29-31).

On the other hand, apparently not all functional proteins associated with the microsomes, such as NADH-cytochrome b reductase (32), turn over rapidly. Certainly the resolving power of the SDS-acrylamide gel electrophoretic technique employed in the present study is not sufficient to conclude that there is complete uniformity of turnover of all membrane proteins. Perhaps the application of recently developed techniques for the two-dimensional electrophoresis of microsomal membrane proteins (33, 34) will provide a clearer indication of the relative rates of turnover of different microsomal membrane proteins.

The question remains as to the reason for the existence of two kinetically distinct classes of endoplasmic reticulum. If the rapid turnover of these membranes is closely related to the intracellular turover of secretory products, it is possible that the dynamic and more stable membrane proteins originate from 2 cell populations that differ according to whether or not they are actively secreting. In this regard, it is of interest to note that under normal nutritional conditions only a small fraction of hepatic parenchymal cells appear to actively secrete albumin and fibrinogen at any single time (35-37). A similar explanation has been proposed to account for the biphasic decay of membrane protein in myeloma cells (1).

Finally, the similarity between the rates of decay of the dynamic membrane protein and newly synthesized serum protein associated with the membranes (as shown in Fig. 5, both exhibit half-lives of approximately 1 h) suggests that in liver, as has been proposed recently for the myeloma cell (1,2), the rapid synthesis and degradation of the membranes may be the driving force for membrane flow which, in turn, has been proposed to serve as the driving force for the intracellular transport of secretory products (38, 39).

Acknowledgments—We wish to thank Drs. Edward Khairallah and Beth Genya Cohen of this department for their assistance in preparing this manuscript.

REFERENCES

Rapid Turnover of Hepatic Endoplasmic Reticulum