Intracellular and Transcellular Transport of Secretory Component and Albumin in Rat Hepatocytes

ELIZABETH S. SZTUL, KATHRYN E. HOWELL,* and GEORGE E. PALADE

Section of Cell Biology, Yale University School of Medicine, New Haven, Connecticut 06510; and
*European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany

ABSTRACT The intra- and transcellular transports of hepatic secretory and membrane proteins were studied in rats in vivo using [3H]fucose and [35S]cysteine as metabolic precursors. Incorporated radioactivity in plasma, bile, and liver subcellular fractions was measured and the labeled proteins of the Golgi complex, bile, and plasma were separated by SDS PAGE and identified by fluorography. [3H]-radioactivity in Golgi fractions peaked at 10 min postinjection (p.i.) and then declined concomitantly with the appearance of labeled glycoproteins in plasma. Maximal secretion of secretory fucoproteins from Golgi occurred between 10 and 20 min p.i. In contrast, the clearance of labeled proteins from Golgi membrane subfractions occurred past 30 min p.i., indicating that membrane proteins leave the Golgi complex at least 30 min later than the bulk of content proteins. A major 80,000-dalton form of secretory component (SC) was identified in the bile by co-precipitation with (IgA)2 by an anti-IgA antibody. An antibody (raised in rabbit) against the biliary 80,000-dalton peptide recognized two larger forms (116,000 and 94,000 dalton), presumably precursors, in Golgi membranes. A comparative study of kinetics of transport of 35S-SC and 35S-albumin showed that albumin peaked in bile at ∼45 min p.i., whereas the SC peak occurred at 80 min p.i., suggesting that the transit time differs for plasma and membrane proteins that are delivered to the bile canaliculus.
in the bile; and (c) the nature and origin of these biliary proteins.

With the exception of albumin, practically all plasma proteins are glycoproteins and many of them are fucosylated (2). SC is also fucosylated and so are other biliary secretory glycoproteins. Hence, \[^{[3]H}\]fucose can be used, in addition to radioactive amino acids, for the comparative studies mentioned above.

In this paper we report data obtained using \[^{[3]H}\]fucose and \[^{[35]S}\]cysteine in a study of the pathways and kinetics involved in the transport of cohorts of secretory and membrane proteins to the sinusoidal and biliary aspects of hepatocytes. We intend to use this information as background against which to study the kinetics and transport pathways of the SC from its site of synthesis to its final discharge into bile.

MATERIALS AND METHODS

Materials

Specific biochemical compounds were purchased from Sigma Chemical Co. (St. Louis, MO); \[^{[6-3H]}\]fucose, 27 Ci/mmol and \[^{[35]S}\]cysteine, 1,080 Ci/mmol were purchased from Amersham Corp. (Arlington Heights, IL). Na\(^{251}\) was obtained from New England Nuclear (Boston, MA).

Methods

In Vivo Labeling Experiments

120–180-g male Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, MA) were anesthetized with intraperitoneally injected Nembutal (Abbott Laboratories, North Chicago, IL) at 0.1 ml/100 g animal wt, and received the label by injection into the saphenous vein. At various times after label administration, the livers were removed for further processing.

Sampling of Blood and Bile

Blood was collected (under anesthesia) from the tail vein and allowed to clot. The serum was separated from the clot by low speed centrifugation. Bile was collected (under anesthesia) via a canula (PE-50 tubing, Clay Adams, Piscataway, N J) inserted into the common bile duct.

Liver Homogenization and Cell Fractionation

Livers were removed from anesthetized rats, placed in ice-cold 0.25 M sucrose, and minced. The mince was washed free of blood and then homogenized with a motor-driven Teflon pestle in a Bredtler homogenizer to give ~30% wt/vol homogenate.

Golgi fractions were isolated by modification (12) of the procedure of Ehrenreich et al. (7) omitting in all cases ethanol administration to the animals.

Three Golgi fractions were obtained: GF\(_{1-2}\), GF\(_{1m}\), and GF\(_{3}\). GF\(_{1-2}\) was defined as the material that floated to the 0.25 M/0.86 M sucrose interface. As previously documented (12), this fraction contains predominantly vesicles filled with lipoprotein particles; Golgi cisternae are present as a minority component. GF\(_{1m}\) was collected at the 0.86 M/1.15 M sucrose interface. This fraction contained predominantly cisternal elements, in agreement with findings already published (7). GF\(_{1m}\) fraction elements partitioned into 0.86 M sucrose and consisted of cisternae in roughly the same number as vesicular structures.

Subfractionation of the Golgi Fraction

A total Golgi fraction, consisting of pooled GF\(_{1-2}\), GF\(_{1m}\), and GF\(_{3}\), was subjected to alkaline treatment, as in (12), followed by centrifugation to separate Golgi content and Golgi membrane subfractions.

Gel Electrophoresis

SDS PAGE was carried out as described (12), except that a linear 5–15% polyacrylamide gradient and a 1.5-mm-thick slab gel apparatus were used. Electrophoresis was carried out at room temperature at a constant current of 35 mA for ~9 h. Samples of serum, bile, and liver subcellular fractions were prepared for electrophoresis as in (12), except that bile samples (200 μl) were treated with 2 ml of ice-cold acetone to precipitate proteins; the latter were resuspended in electrophoresis buffer prior to boiling. Gels were processed for fluorography with EN\(^{3}\)HANCE (New England Nuclear).

Radioiodination

Peptides to be radioiodinated (10–50 μg) were mixed with carrier-free Na\(^{125}\)I (2 mCi), and 0.5 M sodium phosphate buffer, pH 7.2, in a final volume of 100 μl. The mixtures were transferred into tubes plated with Iodo-gen (Pierce Chemical Co., Rockford, IL) and the reaction was allowed to proceed for 10 min on ice. Free Na\(^{125}\)I was separated from labeled peptides by desalting on Sephadex G-25 medium (Pharmacia Fine Chemicals Div., Pharmacia Inc., Piscataway, NJ) columns. Rat albumin was labeled to a specific radioactivity of 0.30 mCi/10 μg albumin (1.2 x 10\(^{4}\) cpm/μg), while rat bile proteins contained 7.06 x 10\(^{4}\) mCi/200 μg protein (1.47 x 10\(^{5}\) cpm/μg).

Immune Precipitation

Samples were made 2% in SDS, boiled 2 min, and made up to a final concentration of 2% Triton X-100, 150 mM NaCl, 2 mM EDTA, 30 mM Tris-HCl, pH 7.6 (immunoprecipitation buffer). To each sample 50 μl of sheep anti-rat IgA serum (alpha chain specific) (Cappel Laboratories, Inc., Cochranville, PA) was added, and antigen-antibody complexes were allowed to form overnight at 4°C. Immune complexes were adsorbed on protein A-Sepharose (Pharmacia Fine Chemicals) as follows: to each sample, 60 μl of a solution consisting of 50% beads/50% immunoprecipitation buffer was added, and the mixture was incubated for 1 h at room temperature. The beads were sedimented, washed three times with immunoprecipitation buffer, two times with the same buffer without Triton X-100, and processed for SDS PAGE as described above.

Analytical Procedures

Radioactivity Determination: An equal volume (1 ml) of ice-cold 1% phosphotungstic acid (PTA) in 0.5 M HCl was added to each sample of immunoprecipitate. The precipitate was washed three times by resuspension in the same PTA solution followed by low speed centrifugation and the final pellet was resuspended in 0.5 ml of 1% SDS. After addition of Liquiscint (National Diagnostics, Inc., Somerville, NJ), the samples were counted in a Beckman LS-250 liquid scintillation system (Beckman Instruments, Palo Alto, CA). Lipid Extraction: Lipids were extracted from cell fractions according to Saito and Hakamori (34), first with 20 vol of chloroform/methanol (2:1), and then with 20 vol of chloroform/methanol (1:2). The organic phases were pooled, dried under N\(_2\), and the residue was redissolved in chloroform. Chloroform solution samples were counted upon addition of Liquiscint.

Protein Assay: Protein was estimated using the Bio-Rad Protein Assay (Bio-Rad Laboratories, Richmond, CA), with BSA as the standard.

RESULTS

Clearance of \[^{[3]H}\]Fucose Radioactivity from the Blood Plasma

Since the interpretation of our kinetic data depends critically on pulse-chase conditions, and all our experiments were carried out on intact animals, we had to determine beforehand the rate at which radioactivity is cleared from the blood plasma after an intravenous \[^{[3]H}\]fucose injection (0.5 mCi, 0.01 μmol). The data obtained (Fig. 1A) indicated that ~90% of the label was removed from circulation within 3 min postinjection (p.i.); only 5% was still present at ~10 min; and none was detected past 20 min. These values refer to acid-soluble radioactivity, presumably free \[^{[3]H}\]fucose, and were the same for either 0.5 or 1.0 mCi of radioactivity initially administered. Clearance was slightly delayed when

\(^2\) PTA/HCl precipitation was used because it was found to be more efficient than trichloroacetic acid in precipitating glycoproteins.

\(^3\) For convenience, we used serum rather than plasma for all such determinations. Since, for our purpose, the two can be considered equivalent, they will be referred to hereafter as plasma samples and plasma proteins.
the dose was raised to 1.5 mCi [3H]fucose, a concentration used in some experiments (see figure legends) in which high levels of labeling were necessary. 

[3H]fucose is, therefore, cleared rapidly enough from the blood to give acceptable pulse-chase conditions, and to make possible (within the time limits indicated) kinetic studies on living, intact animals.

**[3H]Fucose Incorporation in the Liver**

The kinetics of [3H]-radioactivity uptake by the liver was investigated by measuring PTA-precipitable counts (expected to represent [3H]fucose incorporated into glycoconjugates, primarily glycoproteins) in whole liver homogenates. The results showed (Fig. 1B) that [3H]fucose incorporation reaches a relatively sharp peak at ~10 min p.i., which is followed by a rapid decline to half-peak values over the next 15 min. They also gave an indication of the relatively small fraction of the initial blood plasma radioactivity incorporated into hepatic glycoconjugates: 0.7, 3.2, 1.6, and 1.3% per gram liver at 3, 10, 20, and 60 min p.i., respectively.

**Kinetics of Secretion of [3H]Glycoconjugates into the Blood Plasma**

Samples of plasma collected during the clearance experiments were processed (by PTA precipitation) to separate free radioactivity from radioactivity already incorporated into glycoconjugates. The results, given in Fig. 1A, showed negligible amounts of radioactivity (probably due to contamination by free [3H]fucose) in samples collected before 10 min. Past 10 min, there was a rapid increase in PTA-insoluble radioactivity, and by 30 min a plateau was reached that continued up to ~110 min.

An examination of Fig. 1 shows that the peaks of the three curves followed each other in the sequence: soluble [3H]-radioactivity (free [3H]fucose) in the plasma at <1 min p.i., [3H]glycoconjugates in the liver at ~10 min p.i., and [3H]-glycoconjugates in the plasma at ~30 min p.i., and shows that the loss of label from the liver, which apparently started at ~10 min p.i., was concomitant with the accumulation of [3H]glycoconjugates in the plasma. These findings are in general agreement with published data (1, 25, 28) that have established, using other metabolic precursors, that most plasma proteins are produced by the liver. Since relevant data on hepatic fucosylated glycoproteins are still limited, we decided to analyze, by SDS PAGE followed by fluorography, the kinetics of appearance of [3H]glycoconjugates in the blood plasma and to compare them with similar kinetics obtained for all serum proteins by using [35S]cysteine as a precursor.

**Survey and Kinetics of Secretion of [3H]- and [35S]-labeled Proteins into the Plasma**

Plasma samples, processed as given under Materials and Methods, contained fucosylated proteins, all of which were radio labeled and detectable past ~20 min (major band) or ~25 min (minor bands) p.i. (Fig. 2A). Maximum labeling was obtained by 30–40 min p.i. and remained at about the same level until the end of the experiments. When [35S]cysteine was used as a precursor (Fig. 2B), labeled proteins were detected in the plasma past ~25 min p.i., reached a maximum at ~50 min p.i., and then plateaued. The 10-min lag between the plateau of [3H]-glycoconjugates (~40 min) and the plateau of [35S]-proteins (~50 min) in plasma represents the minimum time required for the synthesis of secretory polypeptides and for their intracellular transport from the endoplasmic reticulum to the Golgi subcompartment in which fucosylation takes place, plus the time required for terminal glycosylation.

**Appearance of [3H]Fucose Radioactivity in Bile**

Since bile contains both plasma-derived and cell-derived proteins, we decided to examine the kinetics of secretion of [3H]-labeled proteins into bile. To this end, we processed sequentially collected bile samples and examined them for their content of PTA-soluble and PTA-insoluble radioactivity. The results are given in Fig. 3.

PTA-soluble radioactivity, presumably free [3H]fucose, peaked at ~10 min p.i., then decreased progressively at a rather irregular rate until the end of the experiment (3 h p.i.) without ever reaching the baseline. At early time points, [3H]fucose was expected to come directly or indirectly from
FIGURE 2 Appearance of radiolabeled plasma proteins after intravenous injection of label. Blood was sampled as described in Materials and Methods. Approximately 150 μg protein per well was loaded at each time point. (A) samples collected after labeling with 1 mCi [3H]fucose. Lanes 20-50: fluorograph of gel; lane CB: Coomassie Blue staining of the same gel. (B) Samples collected after labeling with 2 mCi [35S]cysteine. Lanes 20-55: fluorograph of gel; lane CB: Coomassie Blue staining of the same gel. (Molecular mass standards, × 10⁻³.)

the blood plasma, but at later time points its source remained unknown. It could not be the plasma, since no free label was detected therein past 60 min p.i., and it was unlikely to be a free [3H]fucose pool in hepatocytes. The late acid-soluble radioactivity might be derived from newly synthesized [3H]-glycoconjugates degraded in hepatocytes by lysosomes or in the bile by discharged lysosomal hydrolases.

Incorporated (PTA-insoluble) radioactivity began to appear in the bile past 20 min p.i., increased rapidly up to ~50 min p.i., remained at a high plateau for the next 30 min, and then decreased gradually during the rest of the experiment.

A comparison of Figs. 1 and 3 showed that ~10 min elapsed between the appearance of [3H]glycoconjugates in the plasma and the emergence of labeled proteins in the bile. The difference increased to ~20 min when peak values or half maximal values were considered.

Survey of 3H- and 35S-labeled Biliary Proteins

As for the plasma, the inquiry was extended to bile proteins resolvable by SDS PAGE. Bile samples, collected sequentially after [3H]fucose administration and analyzed by SDS PAGE followed by fluorography, contained a relatively large number of [3H]-fucosylated glycoconjugates (Fig. 4 A). Three major species of 80,000, 70,000, and 40,000 daltons, respectively, were present in addition to ~8 minor components. The major components became detectable by fluorography at ~25 min p.i. and reached peak labeling at ~80 min p.i. The 80,000-dalton species was identified as the major form of the proteolytically processed SC, since it could be precipitated by an anti-rat IgA serum raised in goats (Fig. 5). Published data (17) indicate that a substantial fraction of the biliary SC is still complexed to (IgA)². The identification of the 80,000-dalton band as SC is in agreement with data published for SC in rabbit milk and bile (15).

A similar survey carried out after [35S]cysteine administration revealed additional, nonfucosylated bile proteins (Fig. 4 B), among which albumin was identified by immunoelectrophoresis at the expected electrophoretic mobility (data not shown). [35S]Albumin reached half maximal value in the bile at ~35 min p.i., ahead of the [35S]80,000-dalton species by ~25 min. Since [35S]cysteine labeling allows estimation of time required for the entire series of operations from polypeptide synthesis to final appearance in the bile, the values mentioned above indicate that the series of modifying and transporting operations that lead to final discharge into bile require about 25 more minutes for SC than for albumin.

Biliary albumin is presumably transported across the cells from the plasma, and a comparison of Figs. 2 and 4 indicates that this operation takes <10 min. Direct evidence for the plasma origin of biliary albumin and for the time needed for its transcellular movement was obtained by injecting 125I-albumin intravenously and following the kinetics of subse-
Figure 4 Fluorograph of SDS PAGE of radiolabeled bile proteins after intravenous injection of label. Bile was sampled as described in Materials and Methods. Approximately 70 μg of protein was loaded per well at each time point p.i. (A) Samples collected after injection of 1.5 mCi [3H]fucose; (B) Samples collected after injection of 1.0 mCi [35S]cysteine. * position of SC, Δ, position of albumin. (Molecular mass standards, × 10^{-3}.)

Figure 5 Identification of the 80,000-dalton bile protein as SC. Total bile proteins were radioiodinated and incubated with either goat anti-rat IgA or goat anti-rabbit IgG antibodies, and the immune complexes were adsorbed on protein A-Sepharose. The immunoadsorbed material was solubilized and analyzed by SDS PAGE. The anti-IgA antibodies were expected to immunoreact with IgA and IgA-SC complexes while the anti-IgG antibodies were included as a nonspecific adsorption control. Lane A, total radioiodinated bile proteins; lane B, like lane A but 1/10 of load; lane C, bile proteins immunoprecipitated with anti-IgG antibodies; lane D, bile proteins specifically immunoprecipitated with anti-IgA antibodies. (Molecular mass standards, × 10^{-3}.)

Summary of Kinetic Data

The kinetic data so far obtained define the following minimal times: (a) <25 min for albumin synthesis, translocation, intracellular transport, posttranslational modifications, and discharge into the plasma (Fig. 2 B); this value is in agreement with data already published (25, 28); (b) <5 min for the transcellular transport of albumin from the blood plasma into the bile; hence, ~30 min for the overall transport operation (Fig. 2 B compared with Fig. 4 B and Fig. 6); (c) 15-20 min for moving newly synthesized secretory proteins from their sites of fucosylation to the plasma (Figs. 1 and 2 A); (d) ~35-40 min for moving SC from its site of synthesis to its site of discharge into the bile (Fig. 4 B); and (e) 25 min for moving SC from its site of fucosylation to the bile (Fig. 4 A).

Still missing from a general kinetic outline of the processes in which we are interested are kinetic data on membrane protein synthesis, glycosylation, and intracellular transport in hepatocytes. Also missing is the identification of the intracellular compartments involved in these various operations, and especially the identification of the compartment(s) in which SC is delayed by reference to albumin.

Presence of ^3H-labeled Plasma Proteins in Hepatic Golgi Fractions

To obtain part of the relevant data, we followed the kinetics of transport of fucosylated proteins through subcellular fractions. The initial experiments were restricted to Golgi fractions for the following reasons: (a) secretory products are...
known to accumulate in Golgi cisternae (primarily dilated rims) and Golgi vacuoles before discharge (1, 28); (b) fucosylation of secretory and membrane proteins appears to occur exclusively in Golgi elements (11, 29, 37); (c) nearly homogeneous Golgi fractions can be isolated from liver homogenates by current procedures; and (d) such fractions can be efficiently resolved into content and membrane subfractions (12).

A partial characterization of the Golgi fractions used in our experiments in terms of the distribution of galactosyltransferase activity and incorporated \([1^\text{H}]\)fucose radioactivity is given in Table I. The two distribution patterns are quite similar when determined at early time points post-\([1^\text{H}]\)fucose administration. The results indicate that fucosylation occurs either in compartments containing galactosyltransferase or in elements co-fractionating with Golgi elements with galactosyltransferase activity (at times later than 30 min p.i., PTA-precipitable \([1^\text{H}]\)fucose did not co-distribute with galactosyltransferase activity, [data not shown]). Additional data concerning these fractions (and the subfractions derived from them) can be found in references 7 and 12.

In the first series of experiments, a Golgi fraction (GF_{1+2}) was isolated (as given under Materials and Methods) 30 min after an intravenous injection of \([1^\text{H}]\)fucose and used to prepare a total content subfraction. The proteins of the latter were analyzed by SDS PAGE, and the ensuing fluorograph was compared with the fluorograph of a blood plasma sample collected at 60 min p.i. Fig. 7 shows that practically all fucosylated plasma proteins are represented in comparable, relative concentrations in the Golgi content subfraction. Since the isolation procedure used takes advantage of the unusually...
low density of hepatic Golgi elements (caused by secretory lipoprotein accumulation) and discriminates against Golgi elements derived from the minority cell populations of the liver, this finding established that the fucosylated proteins of the blood plasma were hepatic products.

Transport Kinetics of \(^{3}H\)-Glycoconjugates in Golgi Fractions

In the following experiments, distinct Golgi fractions, i.e., GF\(_{1+2}\), GF\(_{bet}\), and GF\(_{3}\) were isolated from liver homogenates at selected p.i. times and each fraction was assayed for \(^{3}H\)-radioactivity. The results, normalized per gram liver, are presented in Fig. 8. All fractions reached a labeling peak (of slightly different heights) at \(\sim 10\) min p.i., and then began to lose their label. All three fractions exhibited similar kinetic patterns, but differed in the rates of label clearance; \(^{3}H\)-radioactivity was cleared rapidly from GF\(_3\) and more slowly from GF\(_{bet}\) and GF\(_{1+2}\), the latter being the fraction with the slowest clearance. By 60 min p.i., however, the label in all Golgi fractions was close to base level. The kinetic data in Fig. 8 suggest that \(^{3}H\)fucose was rapidly incorporated in some common elements of the Golgi fractions, most probably Golgi cisternae, and transported promptly and efficiently to elements in GF\(_{bet}\) and especially GF\(_{1+2}\). The curves in Fig. 8 were comparable to the curve in Fig. 1 \(B\) (kinetics of whole homogenate labeling); the difference post-60 min p.i. probably represents label in plasmalemmal proteins or proteins within transcellular, vesicular carriers present in the whole homogenate.

\(^{3}H\)-labeling Kinetics of Golgi Membrane and Content Subfractions

To assess the kinetics of membrane labeling in Golgi elements, Golgi fractions (GF\(_{1+2}\), GF\(_{bet}\), GF\(_{3}\)) were isolated from liver homogenates prepared at selected times post-[\(^{3}H\)]fucose administration (see Fig. 9), pooled, and resolved into a membrane and a content subtraction, as given under Materials and Methods. \(^{3}H\)-radioactivity was determined in each subtraction and the results (normalized per gram liver) are presented in Fig. 9. About 95\% of the label, assumed to represent fucosylated secretory proteins, separated with the content subtraction at 3 and 10 min p.i. Past 10 min p.i., total \(^{3}H\)-radioactivity in the pooled Golgi fractions decreased rapidly (rate of clearance: 2.6\% min\(^{-1}\)) down to \(\frac{1}{2}\) peak value by 60 min p.i.; however, >80\% of the label remained associated with the content subfractions even at 30 and 60 min p.i. \(^{3}H\)-radioactivity in membrane subfractions amounted to a relatively small fraction of the total. It increased rapidly from 3 to 10 min p.i., remained at the same level at 30 min p.i., and decreased (rate of clearance: 1.9 percent min\(^{-1}\)) to slightly less than half maximal value by 60 min p.i. The clearance curve for membrane proteins was, therefore, different from its counterpart for secretory proteins and the egress of fucosylated membrane proteins from Golgi elements appeared signifi-

![Figure 8](image_url)  
**Figure 8** Incorporated label in liver Golgi fractions. Golgi fractions (GF\(_{1+2}\), GF\(_{bet}\), and GF\(_{3}\)) were isolated as described in Materials and Methods from rats sacrificed at various times after intravenous administration of 1.5 mCi [\(^{3}H\)]fucose. The graph shows PTA-precipitable radioactivity normalized per gram liver.

![Figure 9](image_url)  
**Figure 9** Distribution of incorporated [\(^{3}H\)]fucose between Golgi content and membrane subfractions. Golgi fractions were isolated from rats sacrificed at various times after 0.5 mCi [\(^{3}H\)]fucose administration. Pooled Golgi fractions (GF\(_{1+2}\), GF\(_{bet}\), and GF\(_{3}\)) were separated into content and membrane subfractions (as described in Materials and Methods). The graph shows amounts and percent distribution of PTA-precipitable [\(^{3}H\)]radioactivity normalized per gram liver.
significantly delayed: half maximal values (on the descending slopes) were reached at ~45 min p.i. for fucosylated membrane proteins and at ~28 min p.i., for their secretory counterparts.

Since the membrane subfraction accounted for a small percentage of the total Golgi fraction label, and since complete removal of secretory proteins from membranes is known to be difficult (4), we assessed the efficiency of the subfractionation procedure by comparing fluorographs of the content subfractions with fluorographs of the membrane subfractions obtained at different steps in our membrane "cleaning" procedure. The results, presented in Fig. 10, show that after the final step (i.e., gradient centrifugation after carbonate and high salt treatment), most of the content bands originally present in the membrane preparation were removed, and that a number of membrane-specific bands could be recognized. The data indicate that fucosylated glycoproteins are present in Golgi membranes, although these membranes are still detectably contaminated by secretory glycoproteins. It follows that the distribution figures given in Fig. 9 for membrane-associated radioactivity are maximal values, and that the difference in clearing kinetics between secretory and membrane proteins may be greater than indicated above.

Since fucose is present in both glycoproteins and glycolipids, Golgi subfractions prepared at 45 min p.i. from pooled Golgi fractions were extracted as in reference 34 and the partition of 3H-radioactivity between lipids and proteins was determined. The data in Table II indicate that only a small fraction of radioactivity (2% for content and 11% for membranes) could be ascribed to glycolipids. Therefore, our data concern essentially Golgi glycoproteins.

For reasons mentioned in the introduction, SC should be present among the fucosylated glycoproteins in the Golgi membrane subfraction. Antibodies raised in rabbits against rat bile 80,000-dalton SC (manuscript in preparation) were used to separate by specific immunoprecipitation SC from other bile or Golgi membrane proteins. As shown in Fig. 11, a fucosylated band of ~80,000 dalton (corresponding to the original antigen) was immunoprecipitated from bile. Two peptides of 116,000 and 94,000 dalton were immunoprecipitated from a Golgi membrane subfraction. Their relation to SC will be analyzed in a subsequent paper.

![Figure 10](image1.png)

**Figure 10** SDS PAGE analysis of 3H-proteins in Golgi content and membrane subfractions. A Golgi fraction (GF1×2) was isolated from rats sacrificed 20 min after 1 mCi [3H]fucose administration and resolved into content and membrane subfractions as described in reference 12. The membrane subfraction was analysed by SDS PAGE at three different steps of a cleaning procedure. Lane A: the membranes after 100 mM Na2CO3, pH 11.3, treatment; lane B: like lane A but further washed with 0.25 M sucrose, 50 mM Tris-HCl, pH 7.4, 0.5 M KCl, and 5 mM MgCl2; lane C: like lane B but membrane subfraction was centrifuged to equilibrium on a sucrose gradient; lane D: content subfraction. Approximately 55 μg of protein loaded per lane. Arrows indicate proteins selectively removed during membrane cleaning. (Molecular mass standards, X 10^-3.)

![Figure 11](image2.png)

**Figure 11** [3H]fucose-labeled SC in Golgi membranes. A Golgi fraction (GF1×2), isolated from a rat sacrificed 45 min after 1 mCi [3H]fucose administration, was resolved into content and membrane subfractions by treatment with 100 mM Na2CO3, pH 11.3 (as in Materials and Methods). Approximately 200 μg of membrane subfraction protein was taken for immunoprecipitation (as in Materials and Methods) except that anti-rat bile SC serum was used. Bile (~200 μl), collected between 80 and 100 min p.i. of 2 mCi [3H]fucose, was immunoprecipitated using anti-SC serum. Lane A: [3H]fucose-labeled Golgi membrane peptides; lane B: [3H]fucose-labeled Golgi membrane proteins immunoprecipitated with anti-SC serum; lane C: [3H]fucose-labeled bile proteins; lane D: [3H]fucose-labeled bile proteins immunoprecipitated with anti-SC serum. (Molecular mass standards, X 10^-3.)

**Table II** Distribution of 3H-Radioactivity between Glycolipid and Glycoprotein in Golgi Content and Membrane Subfractions

| Golgi subfractions | % |
|--------------------|---|
| Glycoprotein       | 98 | 89 |
| Glycolipid         | 2  | 11 |

**TABLE II** Distribution of 3H-Radioactivity between Glycolipid and Glycoprotein in Golgi Content and Membrane Subfractions

For reasons mentioned in the introduction, SC should be present among the fucosylated glycoproteins in the Golgi membrane subfraction. Antibodies raised in rabbits against rat bile 80,000-dalton SC (manuscript in preparation) were used to separate by specific immunoprecipitation SC from other bile or Golgi membrane proteins. As shown in Fig. 11, a fucosylated band of ~80,000 dalton (corresponding to the original antigen) was immunoprecipitated from bile. Two peptides of 116,000 and 94,000 dalton were immunoprecipitated from a Golgi membrane subfraction. Their relation to SC will be analyzed in a subsequent paper.
DISCUSSION

Some of the secretory proteins produced by hepatocytes, e.g., albumin, appear first in the blood plasma and then in the bile, as a result of two distinct transport operations. The first is carried out along the well studied "secretory pathway" and brings secretory proteins from their site of synthesis to the blood plasma through a series of intracellular compartments (rough endoplasmic reticulum-smooth endoplasmic reticulum-Golgi complex-secretion vacuoles-plasmalemma) (24). Given the information now available, this pathway could be defined as the intracellular rough endoplasmic reticulum-plasmalemma (RER-PL) route (Fig. 12). The second transport operation moves soluble plasma proteins (some of them hepatocytic products) from the plasma to the bile along a transcellular pathway using vesicular carriers of a still unspecified nature.

Integral membrane proteins for the sinusoidal domain of the plasmalemma are also moved along the secretory pathway, but their transport may involve different post-Golgi carriers and different kinetics (36). Integral membrane proteins for the biliary aspect of the plasmalemma may come directly from the Golgi complex or, perhaps indirectly, from the sinusoidal domain of the plasmalemma via the transcellular pathway (18, 27).

SC belongs to a special class of "convertible proteins"; it starts its existence as an integral membrane protein and persists as such at least up to its insertion into the sinusoidal plasmalemma, where it functions as receptor for (IgA)2. With or without its ligand, it is subsequently moved from the sinusoidal to the biliary aspect of the plasmalemma and appears in the bile as a soluble protein. Somewhere along the transcellular pathway, it is physiologically converted (by controlled proteolysis) from an integral membrane protein into a soluble, biliary secretory protein (8, 17).

Our experiments have provided kinetic data on the movement of secretory as well as membrane proteins along both hepatocytic pathways.

Intracellular Pathway (the Rough Endoplasmic Reticulum-Plasmalemma Route)

SECRETORY PROTEINS: Our data refer to the transport kinetics of cohorts of mixed secretory proteins from their site of synthesis to their site of discharge into the blood plasma. In agreement with data already published (1) and as indicated in Fig. 2 B, the minimal time needed for this operation is 20–25 min. Glycoproteins, studied again as a mixed cohort, require a minimum time of 10 min for transport from their site of fucosylation to their discharge into the blood plasma, with discharge continuing for ~20 min (Fig. 13). At the time resolution attained in our experiments, it appears that the fucosylated cohort requires a longer time (~10 min longer) than its nonfucosylated counterpart for intracellular transport and processing. Within the times mentioned, we can fit a Golgi phase (defined only by fucosylation) that starts before 10 min p.i. and ends by ~30 min p.i., overlapping in part with discharge. At present, we do not know whether the individual components of these cohorts are synchronously or asynchronously processed.

MEMBRANE PROTEINS: Our evidence shows clearly that there are fucosylated glycoproteins in Golgi membrane subfractions, that they are rapidly labeled, and that their egress from Golgi fractions—and, by extrapolation, from Golgi complexes in the intact cell—is significantly delayed by comparison with the egress of fucosylated secretory proteins. The lag amounts to ~15 min when half clearance values are considered (Figs. 9 and 13). The secretory component for (IgA)2 is one of the proteins in the cohort of mixed fucosylated membrane proteins we have studied; it has been detected as two immunoreactive bands in Golgi membrane subfractions (Fig. 11), and it will be further characterized in a subsequent paper.

Although the intracellular pathway appears to be reasonably well understood in general outline, many of its details are still poorly defined. For instance, our data refer to fucosylation occurring in the Golgi complex, possibly in Golgi cisternae. But, like galactosyltransferase (and associated enzymes) (32), fucosyltransferase may be restricted in its location to a specific cisterna. Golgi fractions of the type now available cannot provide evidence at this level of resolution. Equally uncertain is the nature of the vesicular carrier that moves fucosylated membrane proteins from the Golgi complex to the plasmalemma. In other cell types, recent studies suggest that this carrier may be different from that transporting secretory proteins (10, 36).

Transcellular Pathway

Published data concerning this pathway in hepatocytes are considerably more limited and fragmentary. The relevant carrier has been identified (by autoradiography [30] and fractionation [22]) as a small, smooth surfaced vesicle of undefined chemistry. Intermediary stations (if any) between the two termini remain unknown but might include endosomal
compartments (5). Our findings provide information limited to (a) the kinetics of transcellular transport of albumin, which appears to be a rapid but quantitatively modest operation (albumin concentration in the bile is ~100-fold less than in the blood plasma [6]; and (b) the kinetics of SC transport over the two combined pathways (Figs. 4 B and 13). The time involved in this operation is substantially longer than the equivalent time for albumin. A delay in SC transport may occur during egress from the Golgi complex; further delays along the transcellular pathway are not excluded. In other systems, soluble proteins and ligand-membrane receptor complexes are taken up by common vesicular carriers and sorted out for different destinations along the transcellular pathway (31). The situation in the hepatocyte is still unknown.

We thank Dr. J. L. Boyer, Liver Study Unit, Yale University School of Medicine for valuable discussion and support during the early phase of this research and Dr. J. D. Castle for useful advice and continuous encouragement throughout the course of this work. We also thank Pamela Ossorio for photography and Cynthia Davis for secretarial assistance.

This work was supported by National Institutes of Health grant GM-27303.

This work constitutes a part of a thesis (E. S. Szul) to be submitted in partial fulfilment for the degree of Doctor of Philosophy.

Received for publication 11 April 1983, and in revised form 18 July 1983.

REFERENCES

1. Bergeron, J. J., J. B. Classen, and J. Cruz. 1978. Passage of serum destined proteins through the Golgi apparatus of rat liver: an examination of heavy and light Golgi fractions. J. Cell Biol. 76:87-97.

2. Blumberg, B. A., and A. L. H. Misono. 1978. Plasma proteins. Wiley-Interscience Div., John Wiley & Sons, Inc., New York. 1-401.

3. Brandtzaeg, P. 1981. Transport models for secretory IgA and secretory IgM. Clin. Exp. Immunol. 44:221-232.

4. Castle, J. D., and G. E. Palade. 1978. Secretion granules of the rabbit parotid. Selective transport of polymeric immunoglobulin A in bile. J. Cell Biol. 79:514-533.

5. Courtour, P. J., J. Quaas, J. N. Lim, C. De Riviére, and P. Baudhuin. 1982. Intracellular sorting of glycosylated proteins and polymeric IgA in rat hepatocytes. J. Cell Biol. 95(2): Pt. 21:425-432 (Abstr.).

6. Delanoeix, D., J. J. H. F. Hodgson, A. McPherson, C. D. W, and J. P. Vaerman. 1982. Selective transport of polymeric immunoglobulin A in bile. Quantitative relationships of monomeric and polymeric immunoglobulin A and immunoglobulin M. other proteins in serum, bile and saliva. J. Clin. Invest. 62:200-241.

7. Ehrenreich, H. J., J. M. Bergeron, P. Stievelitz, and G. E. Palade. 1973. Golgi fractions prepared from rat liver homogenates. I. Isolation procedure and morphological characterization. J. Cell Biol. 59:45-72.

8. Fisher, M. B., M. Nagy, H. Bazin, and B. J. Underwood. 1979. Biliary transport of IgA: role of secretory component. Proc. Natl. Acad. Sci. USA. 76:2008-2012.

9. Fleischer, B. 1974. Isolation and characterization of Golgi apparatus and membranes from rat liver. Methods Enzymol. 31:180-191.

10. Gumbiner, B., and B. B. Kety. 1982. Two distinct intracellular pathways transport secretary and membrane glycoproteins to the surface of pituitary tumor cells. Cell. 28:51-59.

11. Hand, A. R. 1979. Synthesis of secretory and plasma membrane glycoproteins by sialized duct cells of rat salivary glands as visualized by radioautography after 3H-fucose injection. Anat. Rec. 195:317-340.

12. Howell, K. E., and G. E. Palade. 1982. Hepatic Golgi fractions resolved into membrane subfractions. J. Cell Biol. 92:822-837.

13. Jos, J., L. Labbe, B. Gery, and C. Griscelli. 1979. Immunoelectron-microscopic localization of immunoglobulin A and secretory component in jejunal mucosa from children with coeliac disease. Scand. J. Immunol. 9:441-450.

14. Kim, Y. S., J. Perdomo, and J. Nordest. 1971. Glycoprotein biosynthesis in small intestinal mucosa. J. Biol. Chem. 246:5486-5476.

15. Kahan, L. C. J., and J. P. Kraehenbuhl. 1981. The receptor membrane for polypeptide immunoglobulin is structurally related to secretory component. Isolation and characterization of membrane secretory component from rabbit liver and mammary gland. J. Biol. Chem. 256:12400-12405.

16. Lemaire-Coelho, I., G. A. Altamirano, C. Barrango-Acosta, R. Mekyens, and J. P. Vaerman. 1981. In vivo experiments involving secretory component in the rat hepatic transfer of polypeptide IgA from blood into bile. Immunology. 43:261-270.

17. Lemaire-Coelho, I., G. D. F. Jackson, and J. P. Vaerman. 1977. Rat bile as a convenient source of secretory IgA and free secretory component. Eur. J. Immunol. 7:588-590.

18. Louvard, D. 1980. Apical membrane amineopeptidase apres at site of cell-cell contact in cultured kidney epithelial cells. Proc. Natl. Acad. Sci. USA. 77:4132-4136.

19. Morris, T. J., N. Matthews, and J. Rhodes. 1981. Serum and salivary immunoglobulin A and free secretory component in atrophic colitis. Clin. Allergy. 11:561-564.

20. Mostov, K. E., J. P. Kraehenbuhl, and G. Blobel. 1980. Receptor mediated transcellular transport of immunoglobulin synthesis of secretory component as multiplets and larger membranemembrane forms. Proc. Natl. Acad. Sci. USA. 77:7257-7261.

21. Mullock, B. M., M. Dobrota, and R. H. Hinton. 1978. Sources of the proteins of rat bile. Biochem. Biophys. Acta 543:495-501.

22. Mullock, B. M., R. H. Hinton, M. Dobrota, J. Peppard, and E. Orlans. 1976. Endocytic vesicles in liver carry polymeric IgA from serum to bile. Biochem. Biophys. Acta 587:381-391.

23. Mullock, B. M., J. R. Jones, and H. R. Hinton. 1980. Movement of endocytic shuttle vesicles from the sinusoidal to the bile canalicular face of hepatocytes does not depend on occupation of receptor sites. Biochem. Biophys. Acta 637:109-116.

24. Palade, G. E. 1975. Intracellular aspects of the process of protein synthesis. Science 189:347-358.

25. Peters, T. B., W. Fleischer, and S. Fleischer. 1962. The biosynthesis of rat serum albumin, IV. Appearance of albumin through the Golgi apparatus during secretion. J. Biol. Chem. 246:240-244.

26. Poger, M. E., and M. E. Lammt. 1974. Localization of free and bound secretory component in human intestinal epithelial cells. A model for the assembly of secretory component synthesis. J. Exp. Med. 139:629-642.

27. Quaroni, A., A. Kirsch, A. Hercovics, and K. J. Isselbacher. 1980. Surface-membrane biogenesis in rat intestinal epithelial cells at different stages of maturation. Biochim. Biophys. Acta 95:203-205.

28. Rosenthal, D., and D. R. Abrahamson. 1980. Transport of soluble vesicular contents during Ig transport by the intestinal epithelium of the neonatal rat. J. Cell Biol. 92:178-182.

29. Roth, J., and E. G. Berger. 1982. Immunocytochemical localization of galactose/transferase in HeLa cells codistribution with thiamine pyrophosphatase in trans-Golgi cisternae. J. Cell Biol. 93:223-229.

30. Rozando, R. L., and D. M. Fambrough. 1980. Secretion of acetylcholine receptor metabolism: relation to acetylcholine receptor metabolism. Cell 25:595-602.

31. Saito, T., and S. H. Kimura. 1971. Quantitative isolation of total glycoprotein of mouse peritoneal cells. J. Lipid Res. 13:257-259.

32. Steckley, R., A. D. Burnett, and C. Affold. 1980. The immunological measurement of "free" secretory piece and its relationship to local IgA production. Clin. Exp. Immunol. 45:124-130.

33. Streus, G. J. A., and H. F. Lodish. 1980. Intracellular transport of secretory and membrane proteins in hepatoma cells infected by vesicular stomatitis virus. Cell 22:709-715.

34. Sturgess, J. M., E. Minaber, M. M. Mitranic, and M. A. Moscarello. 1973. The incorporation of L-fucose into glycoproteins in the Golgi apparatus of rat liver and in serum. Biochem. Biophys. Acta 31:132.

35. Takahashi, I., P. K. Nakane, and W. R. Brown. 1982. Ultrastructural events in the translocation of polypeptide IgA by rat hepatocytes. J. Immunol. 128:1181-1187.