PASSAGE OF SERUM-DESTINED PROTEINS THROUGH THE GOLGI APPARATUS OF RAT LIVER

An Examination of Heavy and Light Golgi Fractions

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ABSTRACT

The participation of hepatic Golgi apparatus in the intracellular transport of blood-destined proteins has been analyzed using Golgi fractions enriched in *cis* and *trans* components of the Golgi apparatus.

SDS-polyacrylamide gel electrophoresis of the liver Golgi fractions showed several proteins corresponding in relative proportions and mobilities with serum proteins. After a pulse injection of labeled leucine, the secretory content of the *cis* Golgi fraction was labeled earlier than the *trans* Golgi fraction. Taken together, the results show the participation of the liver Golgi apparatus in the secretion of most of the serum proteins and provide documentation for a sequential progression of secretory protein through the *cis* and *trans* components of the Golgi apparatus.

KEY WORDS secretion · serum · subcellular fractionation · gel electrophoresis

The major secretory proteins of the hepatocyte are plasma proteins (7, 10, 11, 15, 20–23, 26, 27), and there is clear evidence for the participation of the hepatocyte Golgi apparatus in the intracellular transport of albumin (10, 26), and VLDL[1] (11).

The object of the present investigation was to assess the intra-Golgi transport of secretory proteins. Thus, rat liver Golgi apparatus were isolated by the method of Ehrenreich et al. (8) which results in three Golgi fractions (light, intermediate, and heavy). Previous morphologic and cytochemical data (8, 9) indicated that the Golgi fractions corresponded to two distinct components of the hepatocyte Golgi complex with the Golgi heavy fraction derived from the *cis* surface of the Golgi apparatus and the Golgi light fraction from the *trans* surface of the Golgi apparatus.[2] We have, therefore, compared the secretory content of the Golgi fractions with the secretory product of the hepatocyte and report a sequential progression of secretory protein from the Golgi heavy to the Golgi light fractions.

[1] *Abbreviations used in this paper:* ER, endoplasmic reticulum; SDS, sodium dodecyl sulfate; VLDL, very low density lipoproteins.

[2] The participation of GERL in the secretory process has been suggested by Novikoff (24). However, whether GERL is a distinct organelle into which secretory protein is transferred directly from the endoplasmic reticulum (ER) and thence packaged into secretory vacuoles (thus bypassing the Golgi apparatus) or whether GERL receives secretory material only after transport through the Golgi apparatus is currently in dispute (12). In any case, the contribution of GERL to the three hepatocyte Golgi fractions has yet to be thoroughly assessed (5, 8, 9).

MATERIALS AND METHODS

All experiments were performed in young (100–140 g) male Sherman rats, starved overnight to deplete intracellular glycogen stores. 1.2 ml/100 g body wt of 50% ethanol was administered via a stomach tube. After a period of 90 min, the animals were lightly anesthetized and injected via the portal vein with 10 μCi/100 g body wt of [^{14}C]leucine (325 mCi/mmol or 283 mCi/mmol) or 100 μCi/100 g body wt of [^{3}H]leucine (5 Ci/mmol), and the animals were sacrificed at various intervals thereafter. Such injected doses were calculated (13, 26) to alter the plasma pool size of leucine by 5.4 and 6.1% for the experiments with [^{14}C]leucine and by 3.5%, with [^{3}H]leucine.

The Golgi light, intermediate, and heavy fractions and a residual microsomal pellet were isolated from liver homogenates by the method of Ehrenreich et al. (4, 8).

The subcellular fractions were characterized and analyzed by the random sampling technique of Baudhuin et al. (2) as modified by Wibo et al. (33), and the ratio of surface area to enclosed volume was estimated for the vesicles and saccules present in each of the three Golgi fractions. This was determined on electron micrographs of the pellicles at 35,000 times magnification. The various elements present in the Golgi fractions were assigned to simple geometric structures, i.e., spheres and collapsed cylinders, and it was assumed that the plane of section went through the midpoint of the structures. The radius and height of the structures (300 chosen randomly) were thus directly determined. Volumes and surface area estimates were thereby made using standard equations.

Subcellular fractions and serum were electrophoresed in 7.5% sodium dodecyl sulfate (SDS)-polyacrylamide gels exactly as described by Bergeron et al. (5), and the proteins were stained with Coomassie Brilliant Blue (Picker Corp., Northford, Conn.). The Coomassie Blue-stained polypeptides were analyzed by means of a converted Leitz cytospectrophotometer constructed by Watzka Ltd., Montreal, and modified by Inventon Reg'd., Montreal, for the analysis of slab gels. Absorbance was determined at a wavelength of 530 nm and recorded on a Honeywell recorder. Fluorography of radioactive gels was carried out according to the procedure of Bonner and Laskey (6).

Radioactivity was determined by precipitation of each of the fractions with ice-cold 5% TCA which contained 0.1 M [^{12}C]leucine. The precipitates were dissolved in 0.2 ml of NCS tissue solubilizer (Amersham/Searle Corp., Arlington Heights, Ill.); after neutralization with acetic acid, 10 ml of Aquasol (Packard Instrument Co., Inc., Downer's Grove, Ill.) scintillant was added and radioactivity was determined in a Packard Tri-Carb model 574. Counts per minute were converted to disintegrations per minute by the use of the channels-ratio and external-standard quench curves as outlined by Wang and Willis (31).

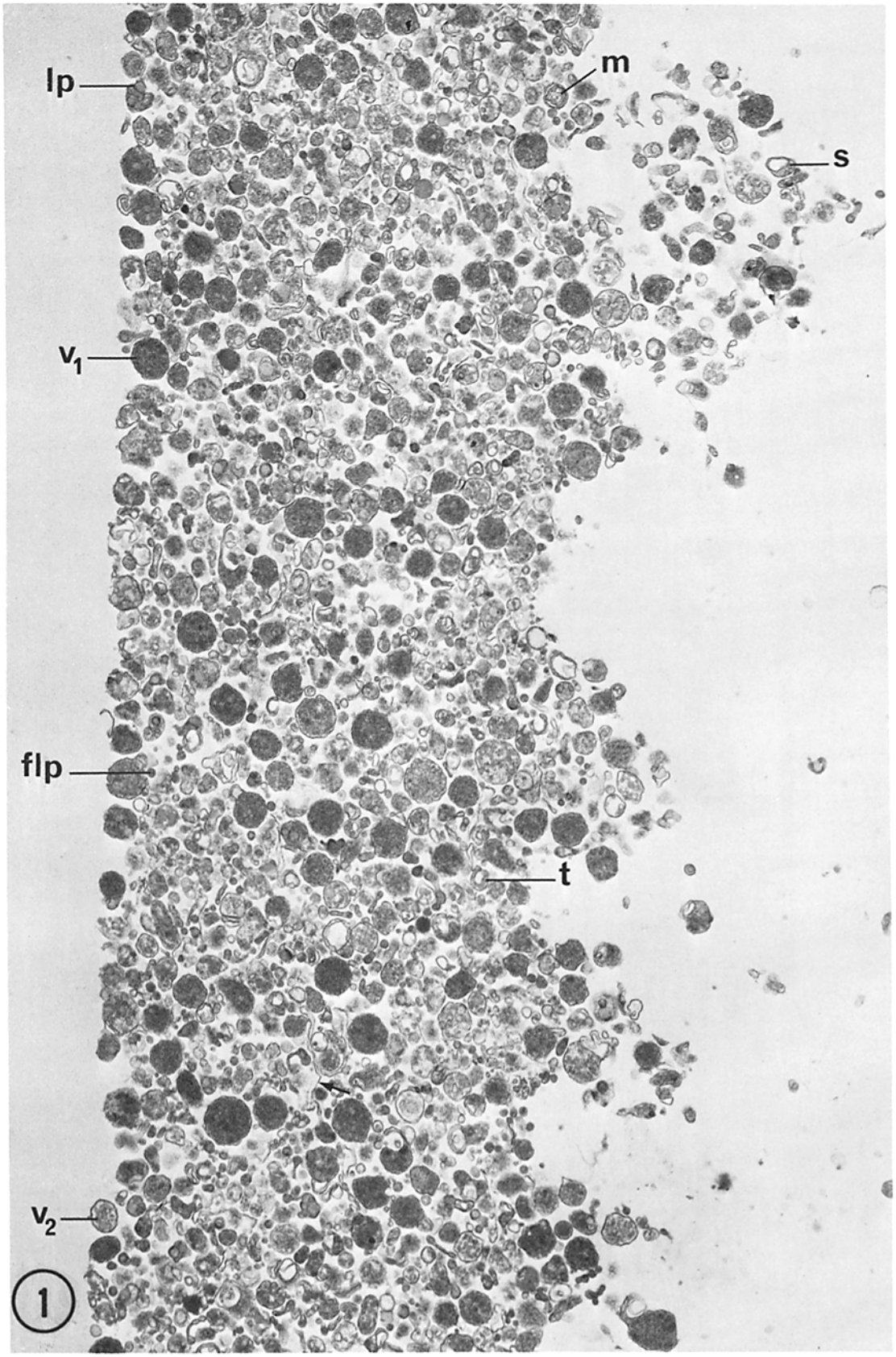
RESULTS

Low-power electron micrographs through the entire depth of the pellicles of the Golgi fractions (Figs. 1, 2) directly illustrate the striking differences in size of the elements making up the Golgi fractions. The Golgi light fraction (Fig. 1) was composed of content-filled vesicles in which the limiting membrane to enclosed content ratio was visibly lower than that of the Golgi heavy fraction (Fig. 2*b*). This latter fraction was composed of flattened sacs with only small amounts of content aggregates located peripherally. A geometric analysis of the structures present in the fractions indicated that the surface area to volume ratios of the Golgi light, intermediate, and heavy fractions were 1:3.1, 1:2.2, and 1.5:1, respectively.

The morphology of part of the pellicle of the residual pellet fraction is illustrated in the higher power electron micrograph of Fig. 3. The fraction consists predominantly of rough ER vesicles, but free ribosomes and smooth-surfaced membranes of unknown origin are also present. The morphology allows one to conclude that the fraction represents a crude rough microsome fraction.

The results of a typical 7.5% SDS-polyacrylamide gel electrophoresis of the three Golgi fractions compared to the residual pellet and the proteins of serum are shown in Figs. 4 and 6. It was possible to distinguish 25 bands in the serum (far right of Fig. 4), 39 bands in the Golgi light

FIGURE 1 Random sample through the entire depth of the pellicle of the Golgi light fraction. The fraction consists mainly of large vesicular elements (v_1) up to 0.5 μm in diameter which is the size of Golgi secretory vesicles found in vivo. The vesicles are all marked by VLDL content (*lp*) although this is more clearly shown at higher magnification (8). An example of a vesicle with a low concentration of content is noted (v_2) as well as free VLDL (*flp*) presumably derived from the opening of Golgi vesicles during the filtration procedure. Irregular structures such as a horseshoe-shaped tubule (*t*) and signet-ring shapes (*s*) are also noted. An example of a membranous structure whose origin is uncertain, i.e., lack of VLDL marker, is noted by an arrow, and a myelin figure is noted (*m*). The pellicle is oriented with the bottom on the left and the top on the right. × 15,000.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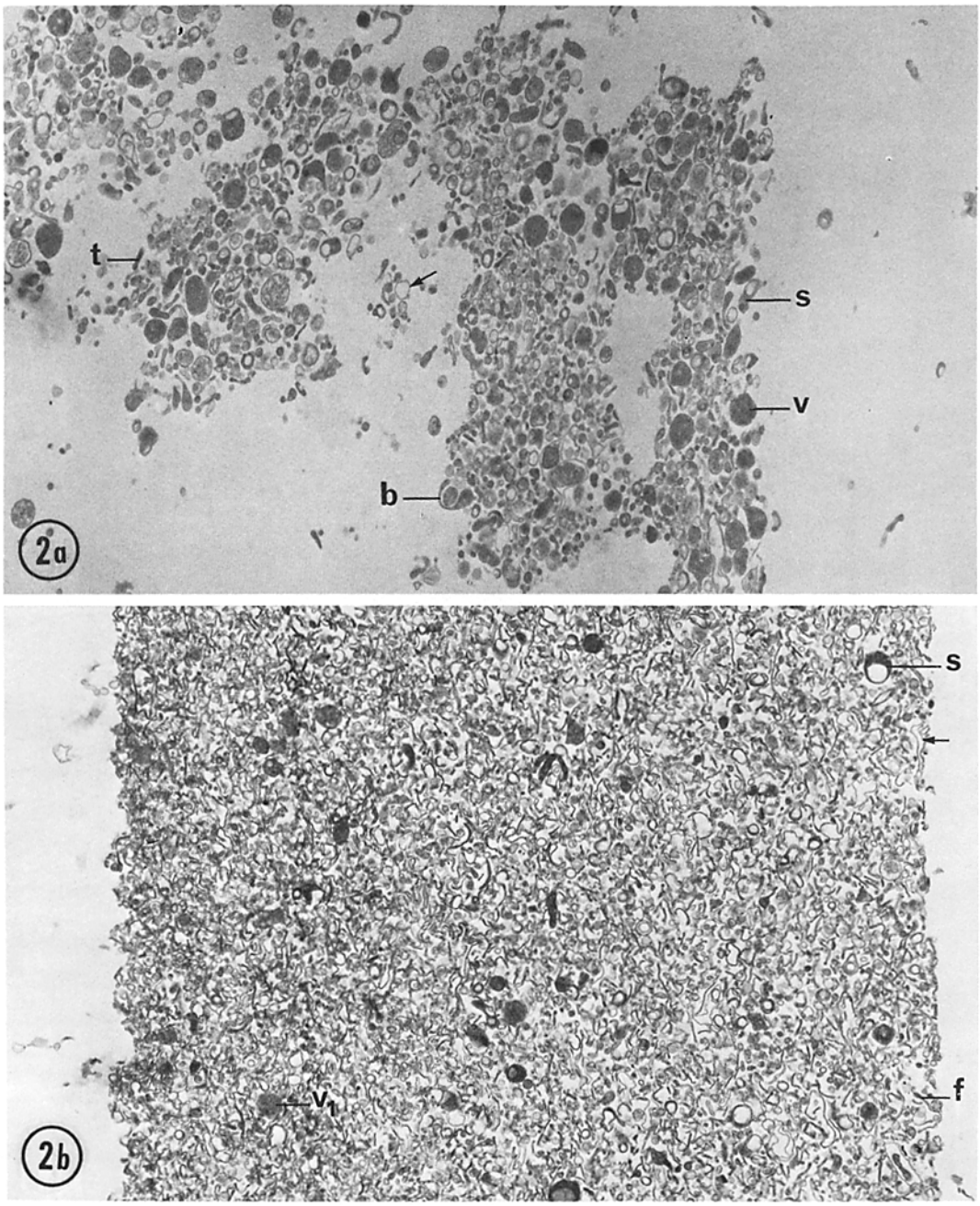

FIGURE 2 (*a*) Random sample of the Golgi intermediate fraction showing that the large vesicles characteristic of the Golgi light fraction are reduced both in number and in size (*v*). The fraction contains a preponderance of irregular-shaped structures such as tubules (*t*), signet-ring shaped structures (*s*), and curved barbell-shaped structures (*b*). Higher magnification shows that virtually all the elments are marked by VLDL content (8), but the rare smooth-surface vesicle (denoted by an arrow) of unknown origin is also present. The bottom of the filtered pellicle is on the right and the top on the upper left. × 12,500. (*b*) Random analysis of the Golgi heavy fraction showing a distribution of elements distinct from the lighter Golgi fractions. The fraction consists predominantly of small collapsed saccules (*f*) 0.2–0.6 μm in length, values smaller than that found for *cis* Golgi elements in vivo. Only the rare Golgi secretory vesicle (v_1) is observed although tubules and signet-ring shapes (*s*) are found. A membranous structure of uncertain origin is noted (arrow). Higher magnification (8) shows that small vesicles often containing a single VLDL are also present in the fraction. The bottom of the filtered pellicle is on the right and the top of the pellicle is on the left. × 12,500.

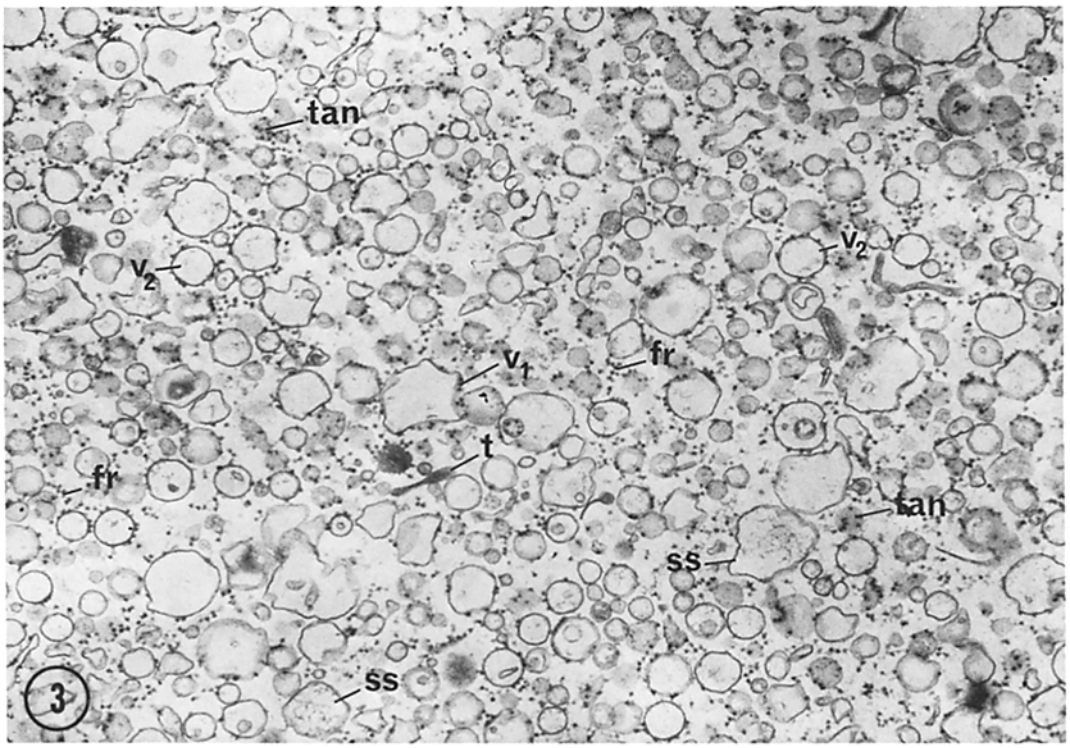

FIGURE 3 Random analysis of the residual pellet fraction viewed in the center of the pellicle. The magnification is higher than that of the previous figures to enable identification of the various elements present in the fraction. The fraction consists predominantly of large irregular vesicles (v_1) and smaller regular vesicles (v_2) studded with ribosomes. Occasional tangential sections (*tan*) also illustrate the ribosomes present on the surface of the vesicles. Free ribosomes (*fr*) are also present, as are rarer smooth-surfaced vesicles (*ss*) and tubular and torus-shaped structures (*t*) probably lysosomal in nature. × 26,100.

and intermediate fractions, 43 bands in the Golgi heavy fraction, and 32 bands in the residual pellet. Certain of these bands were common to all fractions and increased in intensity from the residual pellet fraction → Golgi heavy → Golgi intermediate → Golgi light. Of 23 bands showing this relationship, the bands labeled *5* and *6* (Figs. 4, 6) were the most obvious examples. Of the 23 bands showing increasing intensity, the strongest 14 were correlated with proteins of equivalent mobility (and hence molecular weight) in serum (Table I; molecular weight values determined from Fig. 5), whereas the remaining bands corresponded to very faint bands that were impossible to trace to serum. Densitometry of the gels is shown in Fig. 6, and the results show the peaks representing the most obvious six bands (see Table I) which show an increase in intensity. These six bands correspond in mobility with the six major bands of serum and account for more than 75% (estimated from Fig. 6) of the protein of serum.

The appearance of label in the subcellular fractions is shown in the two experiments of Fig. 7 and two separate experiments on serum as shown in Fig. 9.

The duration of the in vivo "pulse" can be estimated by the time taken for the homogenate to reach a constant specific activity (Fig. 7). Thus, in the experiments on the left of Fig. 7, the pulse duration was less than 5 min and in the experiments on the right of Fig. 7, approximately 10 min. These values agree with estimated clearance times of labeled leucine from the blood (26).

The experiment described on the left of Fig. 7 showed that an increase in the specific radioactivity of protein first occurred in the residual pellet fraction (maximal specific radioactivity at 8 min) followed by that of the Golgi heavy fraction (13 min), the Golgi intermediate fraction (17 min),

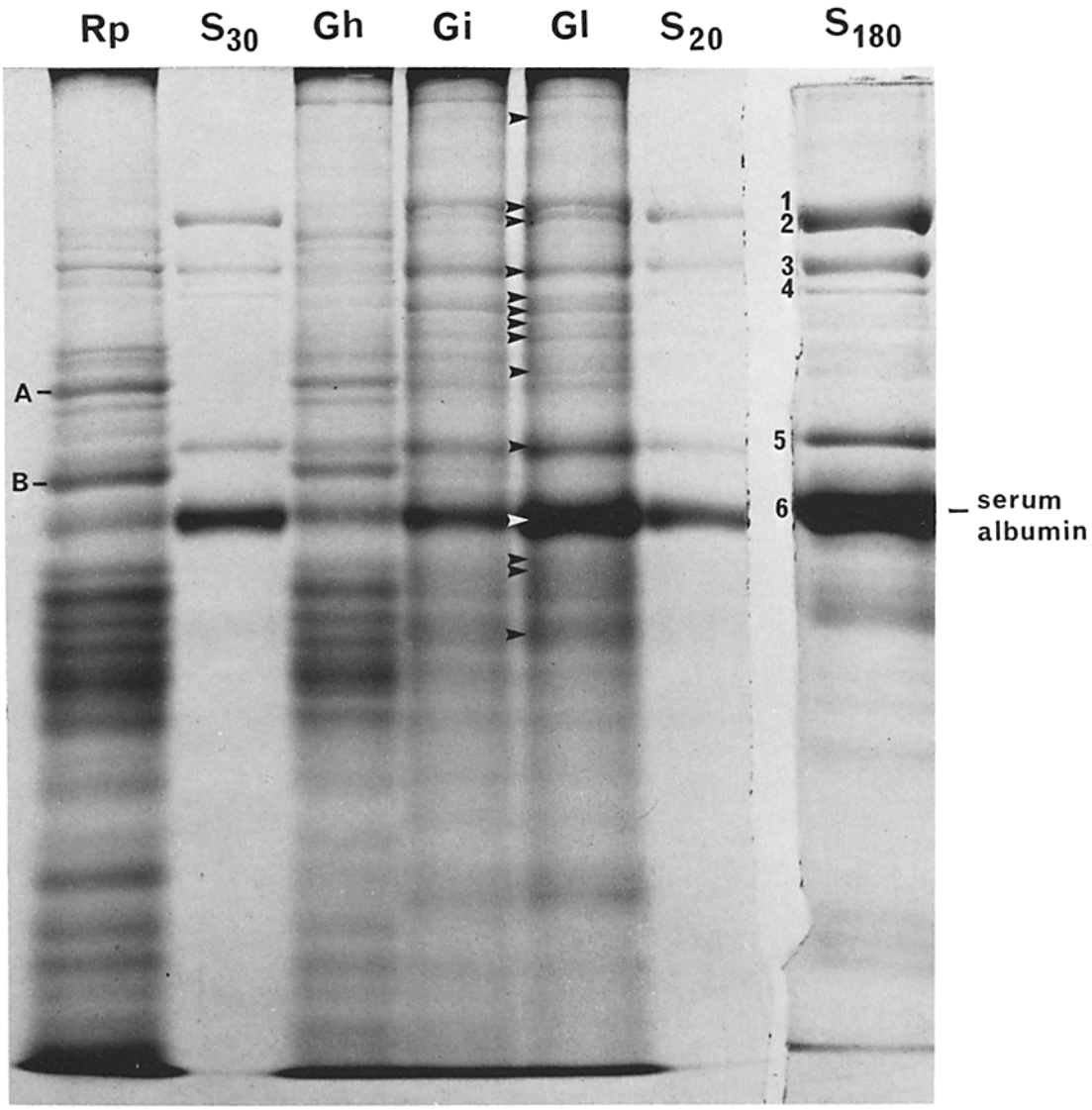

FIGURE 4 SDS-polyacrylamide gel electrophoresis of the Golgi fractions compared to the residual pellet fraction and varying concentrations of serum. From left to right, 250 μg of the residual pellet fraction (R_p), 30 μg of serum (S_{30}), 250 μg of the Golgi heavy (*Gh*), Golgi intermediate (*Gi*), and Golgi light (*Gl*) fractions and 20 μg of serum (S_{20}) were applied to one gel slab. In a separate experiment (far right), 180 μg of serum were applied and electrophoresed (S_{180}) to bring out the weaker staining bands in serum. The major band of the serum co-migrates with rat serum albumin and increases in proportion from the residual pellet to the Golgi light fraction. The bands corresponding to "content" proteins in the Golgi light fraction (*Gl*) are noted by arrowheads. The major bands analyzed in the densitometric analysis of Fig. 6 are numbered in the slot labeled (S_{180}). The two major bands of the residual pellet fraction (*A* and *B*) decrease in proportion from the residual pellet to the Golgi light fraction.

and the Golgi light fraction (20 min). For the experiment shown on the right of Fig. 7, maximal specific radioactivities were found at 8, 13, 20, and 25 min from the residual pellet, Golgi heavy, intermediate, and light fractions, respectively.

A fluorographic analysis of the proteins labeled in the Golgi fractions at the peaks of the specific activity curves is illustrated in Fig. 8. The major labeled protein corresponds to the mobility of albumin.

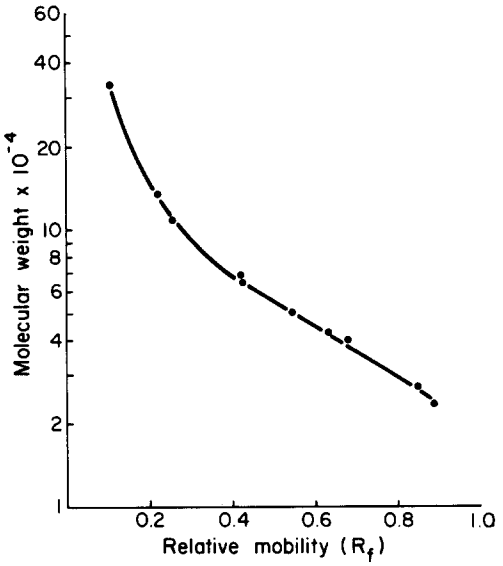

FIGURE 5 Calibration curve of the 7.5% SDS-polyacrylamide gel system. The following molecular weight markers were used: thyroglobulin, 330,000; β-galactosidase, 135,000; collagenase, 109,000; bovine serum albumin, 69,000; rat serum albumin, 64,000; immunoglobulin G (heavy chain), 50,000; ovalbumin, 43,000; aldolase, 40,000; chymotrypsinogen, 25,700; immunoglobulin G (light chain), 23,500.

TABLE I

Molecular Weights of Golgi "Content" Proteins

Mobility (R_f)	Apparent molecular weight	Correspondence to serum bands	Bands labeled in Fig. 6
0.053	$>3.5 \times 10^5$		
0.060	$>3.5 \times 10^5$	+	
0.067	$>3.5 \times 10^5$		
0.084	$>3.5 \times 10^5$		
0.091	$>3.5 \times 10^5$		
0.101	3.5×10^5		
0.137	2.4×10^5	+	1
0.147	2.1×10^5	+	2
0.204	1.5×10^5	+	3
0.228	1.25×10^5	+	4
0.242	1.2×10^5	+	
0.256	1.1×10^5	+	
0.270	1.05×10^5	+	
0.305	9.4×10^4	+	
0.379	7.3×10^4	+	5
0.432	6.4×10^4		
0.446	6.2×10^4	+ (serum albumin)	6
0.498	5.8×10^4	+	
0.505	5.5×10^4	+	
0.566	5×10^4	+	
0.741	3.35×10^4		
0.807	$<3.35 \times 10^4$		
0.821	$<3.35 \times 10^4$		

Relative mobilities of the polypeptide bands of Fig. 4, which progressively increase as a proportion of total protein, are shown in the column headed "Mobility." Apparent molecular weights of these bands were determined by the calibration curve (Fig. 5). The position of the band co-migrating with purified rat serum albumin is indicated. The bands corresponding to the six major bands indicated in Fig. 6 are noted in the column on the right.

As shown in the experiments of Fig. 9, the serum is labeled later (maximal specific radioactivity at 60 and 40 min for the two experiments shown) than any of the liver subcellular fractions analyzed. This is to be expected, as the blood is the site of discharge of the labeled secretory proteins from liver. However, ethanol intoxication is of use in isolating the Golgi fractions (8). The ethanol treatment results in a filling of the Golgi apparatus with VLDL (8, 29, 30) and may thus reflect an alteration in the secretory process of the hepatocyte. However, as shown in Fig. 9, the effect of ethanol intoxication had little effect on either the rate or amount of labeling of secretory proteins.

DISCUSSION

Secretory material synthesized on the ribosomes of the ER is transported via the Golgi apparatus before extracellular discharge (reviewed in reference 25). We have compared five entities to elucidate the sequence of transport in the hepatocyte: (*a*) the residual pellet as representative of what was in the rough ER; (*b*) the heavy, intermediate, and light Golgi fractions as representative of a postulated sequence within the Golgi apparatus; and (*c*) the serum as the site of discharge.

That the transport of secretory material is being considered is based on indirect criteria. Firstly, the polypeptides that increase as a proportion of total protein, in sequentially comparing the subcellular fractions, correspond in molecular weight to the majority of serum proteins. Secondly, there is a rapid labeling of several of these same polypeptides, the most notable example being albumin. Thus, we conclude that we are considering the transport of "content" protein,[3] and the participation of the Golgi fractions in the transport of

[3] Direct attempts to separate membrane from content (8, 16) of hepatocyte subcellular fractions do not result in a complete separation of the two components (17; and unpublished data [J.J.M.B.]).

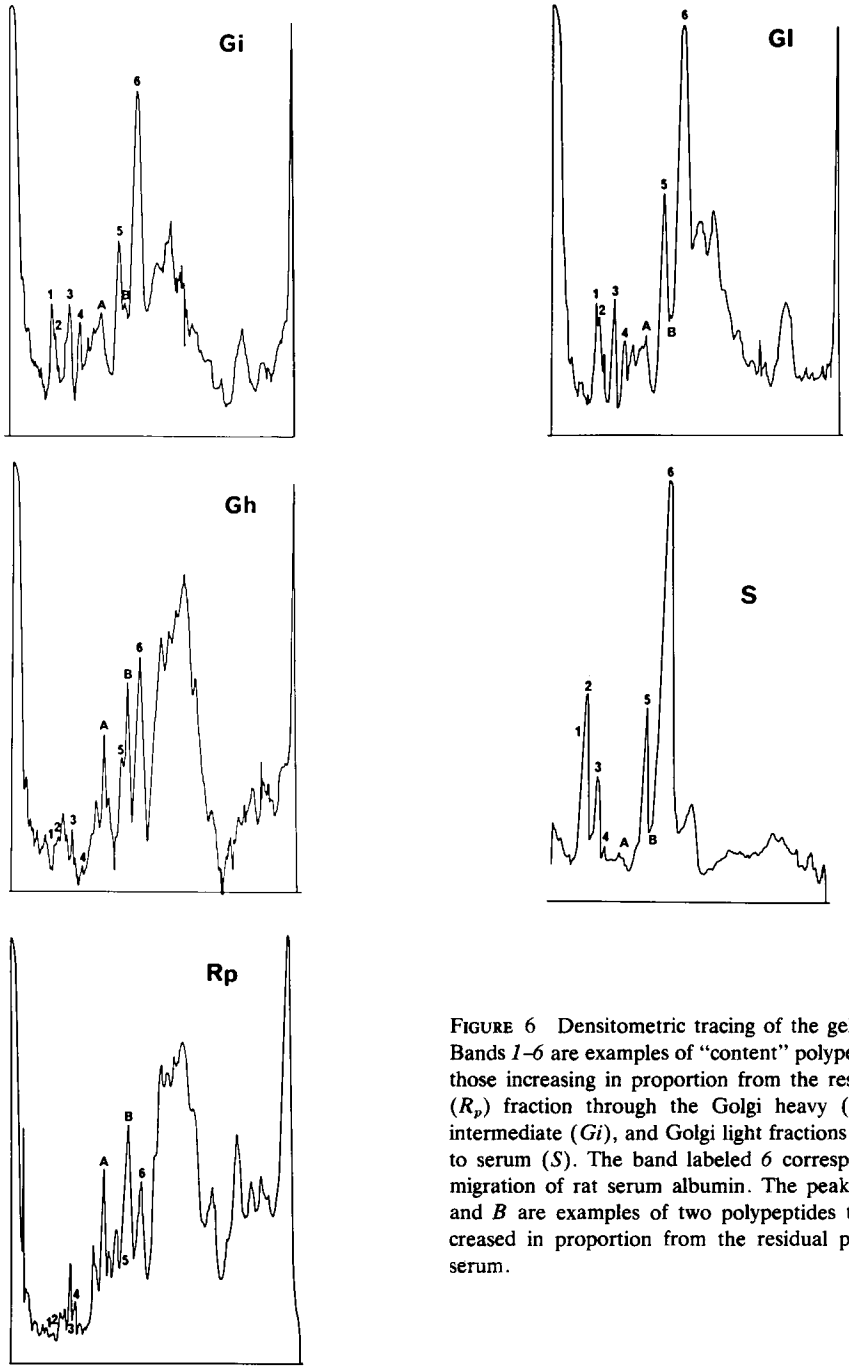

FIGURE 6 Densitometric tracing of the gels of Fig. 4. Bands *1–6* are examples of "content" polypeptides, i.e., those increasing in proportion from the residual pellet (R_p) fraction through the Golgi heavy (*Gh*), Golgi intermediate (*Gi*), and Golgi light fractions (*Gl*) finally to serum (*S*). The band labeled *6* corresponds to the migration of rat serum albumin. The peaks labeled *A* and *B* are examples of two polypeptides that are decreased in proportion from the residual pellet to the serum.

most of the secreted serum proteins now seems established.

In addition, the kinetic data show a temporal relationship in the labeling of the proteins of the Golgi heavy and Golgi light fractions. Thus, the Golgi heavy fraction was labeled earlier and to a lesser extent on a total protein basis than the Golgi light fraction, as would be expected for a sequential transport and concentration of secretory content through the *cis* and *trans* elements of the Golgi apparatus.

That the conditions used for the isolation of the

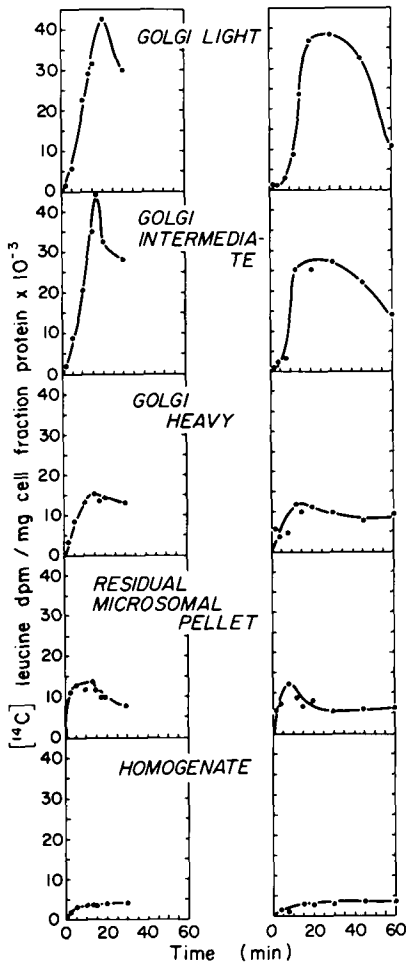

FIGURE 7 Kinetics of transport of labeled protein through the subcellular fractions after intraportal injection of [^{14}C]leucine (10 μCi/100 g body wt). Each point represents the determination of specific radioactivity in fractions isolated from four rats for the experiment on the left and from three rats in the experiment on the right. The experiments on the right and left are identical except that the determinations for the experiment on the right were extended to 60 min. The differences in the timing of the peak specific radioactivities between the two sets of experiments might be due to the "tighter" pulse of 5 min in the experiments on the left as compared with that on the right of about 10 min.

Golgi fractions did not interfere with the normal production of plasma protein was shown by the experiments that compared the rate of labeling of serum proteins in normal and ethanol-intoxicated rats. The ethanol intoxication is known to cause the accumulation of VLDL in the hepatocyte Golgi apparatus up to 4 h after administration (30). The procedure enables the Golgi apparatus to be "floated" away from other intracellular organelles as well as allowing the subfractionation of the Golgi apparatus on the basis of VLDL content (8). Thus, the present data indicate that, contrary to the effect of colchicine (1, 18, 27), ethanol intoxication does not result in a derangement of the secretory process of the hepatocyte (see also reference 14). Other workers have also suggested that alcohol has little inhibitory effect on the secretion of serum lipoproteins from the liver (28, 29). Thus, the accumulation of VLDL in the Golgi apparatus after ethanol intoxication is more probably caused by an increased availability of lipid for secretion. Therefore, data obtained on the membrane constituents (5, 9, 19), as well as those on the secretory proteins (14) of Golgi fractions isolated by the ethanol intoxication procedure, are directly applicable to the functional properties of the Golgi apparatus in the normal rat liver.

The finding of proteins corresponding to the major protein species of the serum in the liver

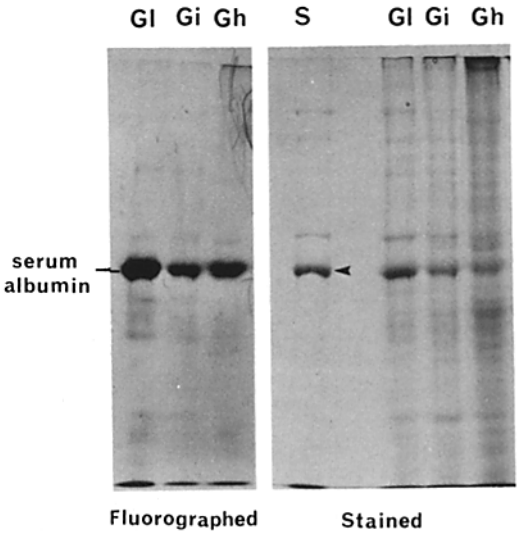

FIGURE 8 Fluorography of labeled proteins of the Golgi fractions. An amount equivalent to 2,000 dpm of the Golgi light (*Gl*) and Golgi intermediate (*Gi*) fractions from the 20-min time-point of Fig. 7 (right) and 2,000 dpm of the Golgi heavy (*Gh*) fraction from the 12-min time-point were electrophoresed, as well as 12 μg of serum (*S*). The Coomassie Blue-stained polypeptides are shown on the right and the image of the fluorographed X-ray film on the left. The position of serum albumin has been indicated by an arrow in the serum slot.

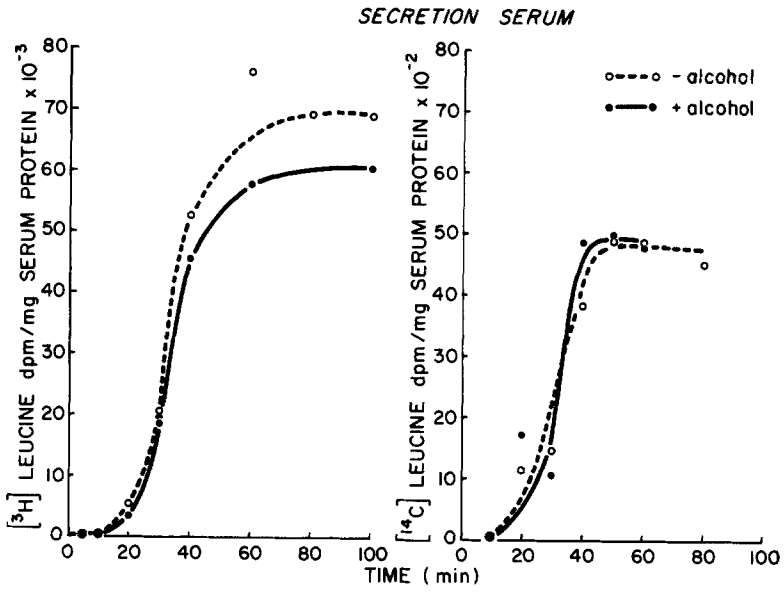

FIGURE 9 Secretion of protein into serum after intraportal injection of labeled leucine. Each point represents the results from two rats after either the injection of 100 μCi/100 g body wt of [^{3}H]leucine (left side of figure) or of 10 μCi/100 g body wt of [^{14}C]leucine (right side of figure). In both instances, the rate of labeling of serum proteins was compared in ethanol-intoxicated (+alcohol) and control (−alcohol) rats. Little difference in the rate or extent of labeling was noted by the prior alcohol treatment. The serum was maximally labeled by 60 min in the experiment on the left and 40 min in the experiment on the right.

Golgi fractions is noteworthy, and the labeling studies are strongly suggestive of a sequential transport of secretory protein through the *cis* and then the *trans* elements of the Golgi elements—a property that has been predicted on anatomical considerations of the Golgi complex (see reference 32 for a review).

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