EVIDENCE FOR THE PARTICIPATION OF THE GOLGI APPARATUS IN THE INTRACELLULAR TRANSPORT OF NASCENT ALBUMIN IN THE LIVER CELL

HANS GLAUMANN AND JAN L. E. ERICSSON

From the Department of Pathology, Sabbatsberg Hospital, Karolinska Institutet, Stockholm, Sweden

ABSTRACT

A comparative biochemical and radioautographic in vivo study was performed to identify the site of synthesis and route of migration of albumin in the parenchymal liver cell after labeling with leucine-^{14}C or leucine-^{3}H via the portal vein. Free cytoplasmic ribosomes, membrane-bound ribosomes, rough- and smooth-surfaced microsomes, and Golgi membranes were isolated. The purity of the Golgi fraction was examined morphologically and biochemically. After administration of leucine-^{14}C, labeled albumin was extracted, and the sequence of transport was followed from one fraction to the other. Approximately 2 min after the intravenous injection, bound ribosomes displayed a maximal rate of leucine-^{14}C incorporation into albumin. 4 min later, a peak was reached for rough microsomes. Corresponding maximal activities for smooth microsomes were recorded at 15 min, and for the Golgi apparatus at $\sim$20 min. The relative amount of albumin, calculated on a membrane protein basis, was higher in the Golgi fraction than in the microsomes. By radioautography the silver grains were preferentially localized over the rough-surfaced endoplasmic reticulum at the 5 min interval. Apparent activity in the Golgi zone was noted 9 min after the injection; at 15 and 20 min, the majority of the grains were found in this location. Many of the grains associated with the Golgi apparatus were located over Golgi vacuoles containing 300–800 A electron-opaque bodies. It is concluded that albumin is synthesized on bound ribosomes, subsequently is transferred to the cavities of rough-surfaced endoplasmic reticulum, and then undergoes migration to the smooth-surfaced endoplasmic reticulum and the Golgi apparatus. In the latter organelle, albumin can be expected to be segregated together with very low density lipoprotein in vacuoles known to move toward the sinusoidal portion of the cell and release their content to the blood.

INTRODUCTION

The endoplasmic reticulum (ER)[1] in the parenchymal liver cell is known to be the site of synthesis of a number of proteins—both serum proteins for export and cellular membrane proteins

[1] Abbreviations used in this paper are as follows: AMP, adenosine monophosphate; ATP, adenosine triphosphate; ER, endoplasmic reticulum; G6P, glucose-6-phosphate; IDP, inosine diphosphate;

(cf. 1, 2). Among the former, albumin is the major component[2]. Other examples of secretory proteins are fibrinogen and different lipoproteins (3, 4). Studies by Peters have demonstrated that the synthesis of albumin occurs at the rough ER and that the nascent protein is subsequently transported to the smooth ER (5). In a series of publications from the Rockefeller University on the synthesis and mechanism of transport of digestive, pancreatic enzymes, the investigators have established the participation of the Golgi region in the route of migration and concentration of these enzymes (6–8). Whether the Golgi apparatus in the liver cell participates in a similar process is not fully understood. In a recent study by Ashley and Peters it was suggested, on the basis of electron microscopic radioautographic analysis of liver slices, that labeled proteins might pass through the Golgi apparatus before being released to the blood (9).

We have performed a comparative biochemical and morphological in vivo study of the site of synthesis and transport of serum proteins, with special emphasis on the role of the Golgi apparatus. Labeling experiments were performed with leucine-^{14}C and leucine-^{3}H. In the biochemical part, albumin was used as a marker for serum proteins. Labeled albumin was isolated from free cytoplasmic ribosomes and from membrane-bound ribosomes, from rough and smooth microsomes, and from Golgi membranes; the biochemical findings were compared with results of electron microscopic radioautography. A preliminary report of this work has appeared (10).

MATERIALS AND METHODS

Animals

Adult male albino rats weighing ~200 g were used. The animals were starved 20–24 hr before sacrifice. In the radioautographic experiments, livers from nonstarved, ~80 g rats were utilized.

Fractionation

PREPARATION OF TOTAL, SMOOTH, AND ROUGH MICROSOMES: Isolation of total microsomes and microsomal subfractions was performed as described earlier (11, 12). Carefully minced livers were homogenized mildly in 0.25 M sucrose in a Teflon-glass homogenizer. A 25% homogenate of liver was centrifuged at 10,000 *g* for 20 min, and the supernate was diluted to restore the original volume. CsCl was added to a final concentration of 15 mM. 8 ml of this supernate was layered over 3.5 ml of 1.30 M sucrose—15 mM CsCl, and centrifuged at 250,000 *g* for 60 min in a Christ Omega II ultracentrifuge (Martin Christ, Osterode am Harz, W-Germany) (rotor 60, tube angle 34°). The fluffy layer at the gradient boundary was collected and diluted with 0.25 M sucrose and centrifuged at 105,000 *g* for 90 min to give a pellet which was designated "smooth microsomes." Since the pellet obtained in the gradient centrifugation had a loose surface, about 2 ml of the 1.30 M sucrose were left behind. Distilled water was added to bring the sucrose concentration to ~0.25 M, followed by centrifugation at 105,000 *g* for 90 min. The resulting pellet was termed "rough microsomes." Total microsomes were prepared as described previously (12).

ISOLATION OF GOLGI APPARATUS: The isolation of a subcellular fraction composed mainly of Golgi elements was, with certain modifications, performed according to Morré et al. (13). 30 g of liver from exsanguinated rats were used for each run. The livers were thoroughly minced in 0.50 M sucrose—5 mM $MgCl_2$. No buffer or dextran was included in any of the media used in this procedure. Homogenization was performed with a Teflon-glass homogenizer at low speed (~100 rpm), with only two complete strokes in order to obtain a mild homogenization. The homogenate was diluted to 90 ml with 0.5 M sucrose and centrifuged at 3000 *g* for 20 min in a Christ Omega II ultracentrifuge (rotor SW 27). The white, upper one-third of the pellet was suspended in 5 ml supernate and diluted to 7 ml with 0.5 M sucrose. The suspension was layered over 22 ml 1.25 M sucrose and was subsequently centrifuged at 60,000 *g* for 60 min in a SW 27 rotor. The white band at the interphase was collected with a Pasteur pipette, diluted with 0.25 M sucrose, and pelleted at 5000 *g* for 30 min. This procedure was repeated once. The pellet was designated "Golgi fraction."

PREPARATION OF RIBOSOMES: Free ribosomes and ribosomes attached to membranes were isolated according to Loeb et al. (14). Only "fraction II" was used for further analysis. The newly synthesized proteins were released from the ribosomes by treatment with puromycin, ATP, and spermine as described by Redman (15).

Washing procedure

In order to dissociate adsorbed proteins, such as hemoglobin and albumin, from the membranes, all

NADH and NADPH, reduced di- and triphosphopyridine nucleotide; PLM, plasma membranes; TCA, trichloracetic acid.

[2] Glaumann, H. 1970. *Biochim. Biophys. Acta.* Vol. 213.

fractions were washed with 0.15 M Tris-buffer, pH 8.0. This treatment does not cause significant rupture of the vesicles or leakage of intracisternal content but efficiently removes adsorbed material[2] (16–18).

Isolation of Albumin

The Tris-washed fractions were resuspended in distilled water, and intravesicular proteins were subsequently released by sonication as described by Campbell et al. (19). $MgCl_2$ was added to a final concentration of 10 mM. Following sedimentation of all membrane fragments at 250,000 *g* for 90 min, the supernate was used for isolation of albumin. The Tris-washed and sonicated pellet was resuspended in 0.15 M KCl—10 mM EDTA and centrifuged at 105,000 *g* for 90 min, and the ensuing pellet was called membrane fraction (20). Albumin was separated from other soluble proteins by utilizing its solubility in TCA-ethanol (21, 22). The sonicated supernate was precipitated with cold TCA to a final concentration of 6%. The pellet was washed in 6% TCA and rinsed with water, suspended in ethanol, diluted with the same volume of water, and was subsequently centrifuged for 30 min at 25,000 *g*. The extraction procedure was repeated once. Aliquots of the ethanol solution were subjected to polyacrylamide disc electrophoresis with rat serum albumin as marker as described in detail elsewhere[2]. The gels were stained with amido schwartz. Only one major band was seen with the same "R_F" value as that of serum albumin, regardless of which subfraction was tested. The albumin was extracted by slicing the sample gel at right angles to the direction of migration, and the slices were homogenized. In order to estimate the recovery of the eluted ablumin from the gels, albumin-^{131}I was added to the TCA-ethanol extracts prior to disc electrophoresis. The recoveries were at an 85% level. When reference is made to amounts of albumin in the following account, corrections are made on the basis of the recovery experiments.

Incorporation of Leucine-^{14}C

DL-Leucine-1-^{14}C (30 mCi/mmole) from The Radiochemical Centre, Amersham, England was injected into a branch of the superior mesenteric vein (5.0 μCi/100 g of body weight). The advantage of portal vein administration in this type of experiment has been demonstrated elsewhere[2]. Radioactivity was measured in a Beckman scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.), DPM-100, with Bray's solution as scintillation mixture (23).

Chemical Analysis and Enzyme Assays

Protein was measured according to Lowry et al. with bovine serum albumin as standard (24). RNA was analysed as before (12). The activities of NADH- and NADPH-cytochrome *c* reductase, G6Pase, IDPase, AMPase, Mg^{++}-ATPase, as well as the amounts of cytochrome b_5 and cytochrome P-450, were estimated as previously described (25). Acid phosphatase was measured according to Bowers et al., with β-glycerophosphate as substrate (26). The assay system for measuring glucosamine transferase activities contained 0.01 M $MnCl_2$, 0.001 M EDTA, 0.03 M Tris-maleate buffer, pH 6.5, 2 mμ-moles UDP-5'-diphospho-N-acetylglucosamine-C^{14} (40,000 dpm), 0.5–1 mg microsomes or 0.2–0.5 mg Golgi membranes in a total volume of 0.1 ml. Incubation was carried out at 37° C for 10 min (27).

Electron Microscopy of Isolated Golgi Fraction

The isolated Golgi fraction was resuspended in 0.25 M sucrose and sedimented at 20,000 *g* for 25 min. Primary fixation was performed in 1.5% cacodylate-buffered glutaraldehyde (pH 7.2) for 12 hr. Following a brief buffer rinse, the pellet was postfixed in 2% collidine-buffered OsO_4 (pH 7.2) for 2–4 hr. The pellet was dehydrated in ethanol and propylene oxide, and was embedded in Epon. Thin sections from different levels of the pellet were stained with uranyl acetate and lead citrate and were examined in a Siemens Elmiskop I electron microscope.

Electron Microscopic Radioautography

5 mCi of L-leucine-4,5-T (500 mCi/mmole; obtained from the Radiochemical Centre) were slowly injected into a branch of the superior mesenteric vein of Nembutal-anesthetized animals. Small biopsies were taken from the liver 2, 5, 7, 9, 12, 15, and 20 min after the beginning of the injection. The tissue was trimmed to cubes, with a side of ~1 mm, under a drop of the fixative (2% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2; or 2% collidine-buffered OsO_4, pH 7.2). The cubes were subsequently immersed in the same fixative for 2 hr (fixation in OsO_4) or 24 hr (fixation in paraformaldehyde). Paraformaldehyde-fixed tissues were briefly washed in cacodylate-buffer and were postfixed in OsO_4 for 2–4 hr. Dehydration and embedding for electron microscopy were performed as described above. The thin sections were picked up on Formvar-coated grids and were stained with uranyl acetate and/or lead citrate. They were then covered first with a layer of carbon by vacuum evaporation and subsequently with fresh Ilford L-4 Nuclear Research

emulsion (Nuclear Research Corp., Southampton, Pa.) according to Caro and van Tubergen (28). The emulsion was exposed for 2, 4, 6, and 7 months, then developed for 90 sec in Kodak D 19 and fixed in Kodak Rapid Fix. The sections were stained with uranyl acetate prior to examination in the electron microscope. Grids lacking sections were used to study the extent of background activity.

RESULTS

Appearance of Labeled Albumin in Subcellular Fractions

A sizable amount of the microsomal protein is not a true component of the membrane but is either adsorbed or contained in the lumen of the vesicles. For this reason, the subfractions were subjected to a washing procedure with alkaline Tris-buffer to dissociate adsorbed protein, and treated with ultrasonication to release the intravesicular content[2] (16–19). Table I shows the amounts of total protein, "nonextractable protein," RNA, and albumin recovered in the Golgi fraction. For comparison, corresponding amounts in the microsomal subfractions are also illustrated. About half of the protein in the Golgi fraction, and also in the microsomal subfractions, could be released by washing with Tris-buffer followed by ultrasonication. The three different types of subcellular fractions isolated, i.e. rough and smooth microsomes, and Golgi elements, all contained albumin. The total rough- and the total smooth-surfaced microsomes contained about the same amount of albumin, or 0.5 mg/g of liver, and the Golgi-rich fraction 0.05 mg. The values for microsomes are somewhat higher than those obtained by Mash and Drabkin (0.59 mg/g in total microsomes) and by Peters (0.36 mg in total microsomes), especially when rough microsomes are compared (5, 29). It is most probable that this discrepancy can be explained by the use of different fractionation procedures. When calculated on a nonextractable protein basis, albumin was found to be more concentrated in the Golgi fraction than in the microsomal subfractions.

In order to evaluate, on a biochemical basis, whether the material isolated in the Golgi fraction originates to any significant extent from contaminating membranes of other cell constitutents, experiments were performed with recognized "marker" enzymes. As is evident from Table II, the Golgi fraction could only contain negligible amounts of plasma membranes and mitochondria since the activities of AMPase and cytochrome *c* oxidase were very low[3]. The Golgi fraction was not entirely lacking in typical microsomal enzymes, such as G6Pase and cytochrome P-450. However, the activities or amounts of these enzymes were only about 25% of the levels found in

[3] There was some activity of acid phosphatase in the Golgi fractions as well as in the microsomal fraction; however, since the enzyme is probably synthesized in the ER and may be transported through the Golgi apparatus, questions concerning the purity of these fractions are hardly answered by measurements of this or other lysosomal enzymes (33).

TABLE I

Protein, RNA, and Albumin in Golgi Fraction and in Microsomal Subfractions

Fraction	Total protein	RNA	Nonextractable protein	Albumin	RNA/ protein	Albumin/ non-extractable protein
		mg protein per g of liver				
Total microsomes	20.1 ± 2.1	4.4	10.2 ± 1.2	0.90 ± 0.2	0.22	0.09
Rough microsomes	10.8 ± 1.2	3.6	5.2 ± 0.6	0.40 ± 0.1	0.33	0.08
Smooth microsomes	7.1 ± 0.8	0.6	3.1 ± 0.3	0.37 ± 0.1	0.08	0.12
Golgi fraction	0.68 ± 0.05	0.06	0.30 ± 0.02	0.05 ± 0.005	0.09	0.17

The values are the means of four experiments ± SEM. Microsomal subfractions and Golgi fraction were isolated as is described in Materials and Methods. For washing the different fractions were twice resuspended in 0.15 M Tris-buffer, pH 8.0, and centrifuged at 105,000 *g* for 120 min. Ultrasonication and isolation of albumin were performed as described in Materials and Methods. Nonextractable protein denotes Tris-washed, sonicated, and KCl-EDTA washed membranes.

Table II

Distribution of Some Marker Enzymes in Different Subcellular Organelles

	Golgi fraction	PLM	Mitochondria	Microsomes
G6Pase*	1.6 ± 0.4	1.2 ± 0.1	0.4 ± 0.1	5.9 ± 0.9
IDPase*	5.8 ± 0.8			19.2 ± 3.0
Mg^{++}-ATPase*	1.9 ± 0.2			1.5 ± 0.2
AMPase*	0.7 ± 0.1	13.5 ± 2.0		1.0 ± 0.1
Acid Pase*	0.3 ± 0.1			0.3 ± 0.1
NADH-cytochrome *c* reductase‡	0.18 ± 0.03			0.67 ± 0.2
NADPH-cytochrome *c* reductase‡	0.016 ± 0.003			0.041 ± 0.01
Cytochrome b_5§	0.10 ± 0.02		0.20 ± 0.05	0.35 ± 0.05
Cytochrome P-450§	0.06 ± 0.03		0.05 ± 0.01	0.45 ± 0.05
Cytochrome *c* oxidase‖	0.10 ± 0.01		3.20 ± 0.05	
Glucosamine transferase¶	0.156 ± 0.03			0.008 ± 0.002

Golgi fraction and total microsomes were isolated as described in Materials and Methods. Mitochondria were prepared according to Ernster and Löw (31) and plasma membranes were separated by the method of Coleman et al. (32). The values are the means of three experiments ± SEM.

* μmoles P_i/20 min per mg protein.

‡ μmoles NADH or NADPH oxidized/min per mg protein.

§ mμmoles/mg protein.

‖ μmoles 0_2/min per mg protein.

¶ mμmoles glucosamine transferred to TCA-insoluble material/10 min per mg protein. Corresponding values for total rough microsomes: 0.004 ± 0.001; and for total smooth microsomes: 0.012 ± 0.004.

total microsomes. Furthermore, in order to investigate more specifically the possible presence of smooth ER in the Golgi fraction, a "mixing" experiment was performed with isolated, leucine-^{14}C-labeled (15 min), total smooth microsomes from 10 g of liver, which were added to a homogenate from the same amount of liver obtained from nonlabeleh rats. A Golgi-rich fraction was separated from the pooled homogenate as described under Materials and Methods. The specific activities of the Golgi fraction only amounted to a few per cent when compared to the label of the original smooth microsomes (not shown in Table II). Also, the small amount of RNA (Table I) seems to rule out significant contamination by rough-surfaced microsomes in the Golgi fraction.[4]

It has been postulated that certain glycosyl transferases are localized in the Golgi apparatus and thus could serve as marker enzymes for this organelle (13, 27, 30). As shown in Table II, glucosamine transferase, when measured with endogenous acceptor according to Wagner and Cynkin (27), although not totally absent from any of the microsomal subfractions was found to be enriched manyfold in the Golgi fraction. Similar findings are reported by Wagner (34).

In summary, the results with marker enzymes and the distribution of RNA as well as the "mixing" experiment demonstrate the relatively high purity of the Golgi fraction isolated by the present method. These results appear to agree well with the morphological observations of the Golgi pellets described below.

For the purpose of clarifying whether the Golgi apparatus participates in the transport of newly synthesized secretory proteins, albumin was extracted from the isolated fraction. Furthermore, free and membrane-bound ribosomes as well as rough and smooth microsomes were separated in order to study and compare the early steps in the synthesis and transport of albumin in the liver cell. In order to achieve an effective and rapid in vivo loading of the liver cell, leucine-^{14}C was administered through the portal vein[2] and the

[4] The presence of RNA in different types of "smooth-surfaced membranes" is a matter of discussion, since theoretically it can be due either to ribosomes, subunits of ribosomes or to "membrane-constituents" (47–49).

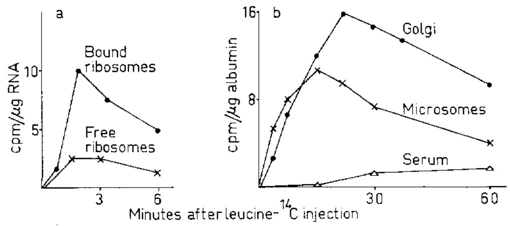


FIGURE 1 Leucine-^{14}C incorporation into albumin of bound and free ribosomes (*a*) and of microsomal and Golgi fractions (*b*). Administration (5 μCi/100 g) and fractionation were performed as described in Materials and Methods. In the experiments with serum, 10 μCi/100 g was administered.

appearance of radioactive albumin in the different cell organelles and in serum was followed. The results of these experiments are summarized in Figs. 1 and 2. As shown in Fig. 1 *a*, the membrane-bound ribosomes at all time points displayed a much higher incorporation rate of leucine into albumin, plotted per mg RNA, in comparison to free ribosomes. A peak of activity was apparent for bound ribosomes approximately 2 min after the administration, followed by a sudden decrease. During the first interval of 10 min, a continuous increase of activity occurred in both total microsomes and the Golgi fraction. However, the specific activity was two to three times higher in the microsomes as compared to the Golgi fraction during this period. A maximum was reached at 15 min for the microsomes, while the Golgi fraction showed a peak value around 20 min.

In Fig. 2, the sequence of appearance of newly synthesized albumin is shown with respect to microsomal subfractions and the Golgi fraction. During the first few minutes after the injection of the amino acid, albumin from rough microsomes displayed an almost fourfold higher rate of incorporation when compared to both smooth microsomes and Golgi fraction, which first paralleled one another but later diverged. Separate localizations of the maxima are apparent, The peak of the rough microsomal fraction was reached after 6 min, that of the smooth fraction after 15 min, and that of the Golgi fraction after ~ 20 min after administration of the amino acid. When the specific activities of the different maxima are compared, that of the Golgi fraction exceeds the activities of the smooth and especially of the rough microsomes.

For the purpose of estimating, quantitatively, the role of smooth ER and the Golgi apparatus in the transport of albumin, Table III depicts total cpm per g of liver as a function against time. At 3 min, the majority of label was recovered in the rough fraction and only 20% in the smooth microsomes. At 9 min, about the same amount was found in the two subfractions. At later time points, the total radioactivity of the smooth microsomes was greater than in the rough counterparts, due to both a decrease in the rough and a simultaneous increase in the smooth microsomes. In comparison with the amount of label in the rough microsomal fraction, the amount of label recovered in the Golgi fraction ranged from a few per cent at the earliest intervals to 15–20% 20 min after the injection of the amino acid.

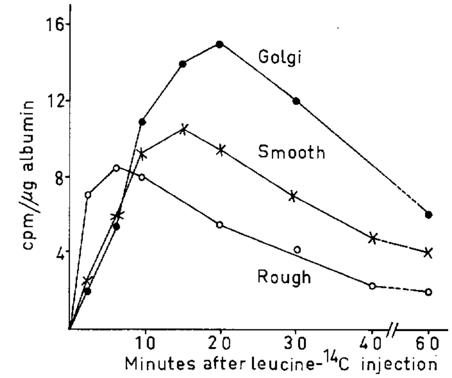


FIGURE 2 Leucine-^{14}C incorporation into albumin of rough and smooth microsomes and of Golgi fraction. Each value represents an average of at least three experiments. Variations from one experiment to another were within 15%.

TABLE III

Distribution of Labeled Albumin in Rough and Smooth Microsomes and Golgi Fraction

Time after injection	Rough microsomes	Smooth microsomes	Golgi fraction
		cpm/g of liver	
3 min	2820	740	68
6 min	3800	2100	150
9 min	3300	3330	370
15 min	2840	3980	470
20 min	2200	3600	520
30 min	1600	2600	410
60 min	870	1480	280

Microsomal subfractions and Golgi fractions were isolated as described in Materials and Methods.

TABLE IV

*Distribution of Radioautographic Silver Grains over Certain Cellular Organelles in Hepatic Parenchymal Cells.**

Cellular organelles	Per cent of total area of selected organelles	Grain concentration at 5 min	at 9 min	at 12 min	at 15 min	at 20 min
		% grains/% area				
Nuclei	16	0.3	0.2	0.3	0.3	0.4
Mitochondria	50	0.1	0.2	0.3	0.3	0.3
RER	20	3.8	1.9	1.5	1.3	0.6
SER Golgi ass.	4	1.6	2.9	3.1	3.1	1.9
Golgi apparatus proper	10	0.3	3.5	4.0	4.7	5.3

* Quantitation of silver grains and distribution of cytological components were performed essentially according to Ashley and Peters (9). The figures given in the Table (only mean values) should be regarded as approximate, since there were comparatively few grains in the sections and the calculations had to be performed on a limited number of negatives (for each interval at least 15 negatives taken at magnifications ranging from 4000 to 6000 and containing from 22 to 43 grains were used). The total number of grains counted was 1874. The calculations were confined to studies of the distribution of the grains in five types of cellular organelles: nuclei, mitochondria, rough-surfaced endoplasmic reticulum (RER), Golgi-associated smooth-surfaced endoplasmic reticulum (SER, Golgi ass.), and the Golgi apparatus proper (vesicles, cisternae, and vacuoles, including Golgi associated "liposomes").

Electron Microscopic Radioautography

In sections exposed for less than 6 months, few grains were present in the tissues. Although these grains were clearly more numerous than in sections investigated for the demonstration of background activity, they were at all intervals too few for valid conclusions. With 6 and 7 months exposure, a definite concentration of grains over certain cytoplasmic organelles was noted 5 min and more after the injection. At shorter intervals, very few grains were seen even with such long exposure times. These grains were located over areas of ground cytoplasm and endoplasmic reticulum; they were unassociated with the nucleus, the mitochondria, and the lysosomes.

The following description pertains to the appearance of hepatic parenchymal cells at intervals ranging from 5 to 20 min after the injection. These tissues showed a low background activity. No clear difference concerning the localization or distribution of grains was observed between OsO_4-fixed and paraformaldehyde-fixed tissues. The distribution of grains over different cytoplasmic organelles is summarized in Table IV.

At 5 min, the majority of grains were concentrated over areas of cytoplasm with endoplasmic reticulum. In some areas there was a tendency toward accumulation of grains over those portions of the endoplasmic reticulum which were located close to the Golgi regions (Fig. 3). Evidence for the presence of significant amounts of radioactive tracer substance inside the Golgi apparatus was not obtained at this interval.

At 9 and 12 min, many grains were found over the different components of the Golgi apparatus, in particular over the vacuoles and the cisternae (Fig. 4). As a rule, the Golgi vacuoles contained large electron-opaque bodies (300–800 A) often referred to as "liposomes." The small size of the vesicular component of the Golgi apparatus made it hard to decide whether the grains were also associated with "Golgi vesicles." Many grains were still located over the endoplasmic reticulum.

15 and 20 min after the injection, there was an even higher concentration of grains over the Golgi area than at previous intervals (Table IV and Fig. 5).

Fine Structure of Golgi Pellets

The appearance of representative areas of Golgi pellets are illustrated in Figs. 6 and 7. The pellets were dominated by the three components of the Golgi apparatus (cisternae, vacuoles, vesicles); however, other cytoplasmic organelles, such as lysosomes, mitochondria, and endoplasmic reticulum, were occasionally seen.

DISCUSSION

The experiments described in this paper aim to explore the route of migration of serum proteins from their site of synthesis to their release into the blood. Special interest was focused on the possible participation of the Golgi apparatus in this process.

Of the different subcellular organelles isolated, all are well characterized with the exception of the Golgi apparatus. The method of isolation of this organelle was based on the same principles as those described by Morré et al. (13). However, in order to avoid complete disruption of the Golgi apparatus into smooth-surfaced vesicles (which results in the obliteration of its specific morphological features), homogenization with a Polytron homogenizer (Brinkmann Instruments, Westbury, N. Y.) was omitted. Instead, mild homogenization was performed with a Teflon-glass homogenizer. With the method described, the isolated Golgi fraction was shown to consist of (*a*) flattened, often multiple and parallel cisternae, (*b*) vacuoles containing electron-opaque material, and (*c*) numerous small peripheral vesicles. Identical morphological features are exhibited by the Golgi apparatus *in situ* (see reference 35).

Fine structural analysis of the Golgi pellets revealed only slight contamination with other subcellular organelles, mainly microsomes. The comparatively high purity of the fraction was also indicated by the results of the enzyme assay. The enrichment of glucosamine transferase activities in the Golgi profiles is in line with the results of Morré et al., and Wagner and Cynkin, indicating that this enzyme, as well as UDP-galactose: N-acetyl-glucosamine galactosyl transferase, as demonstrated by Fleischer et al., might be useful as marker for the Golgi apparatus (13, 27, 30). On the other hand, data obtained by Molnar et al. demonstrated that total rough and total smooth microsomes are active in hexosamine and mannose transfer reactions (36). It is interesting to note in this connection that within both the smooth and the rough microsomal fractions there exists an appreciable heterogeneity as regards enzymic distribution in different types of vesicles (18, 25, 37, 38). Whether this is also true for enzymes participating in the synthesis of carbohydrate side-chains of glycoproteins—thereby explaining some of the contradictory results in the literature—remains to be investigated.

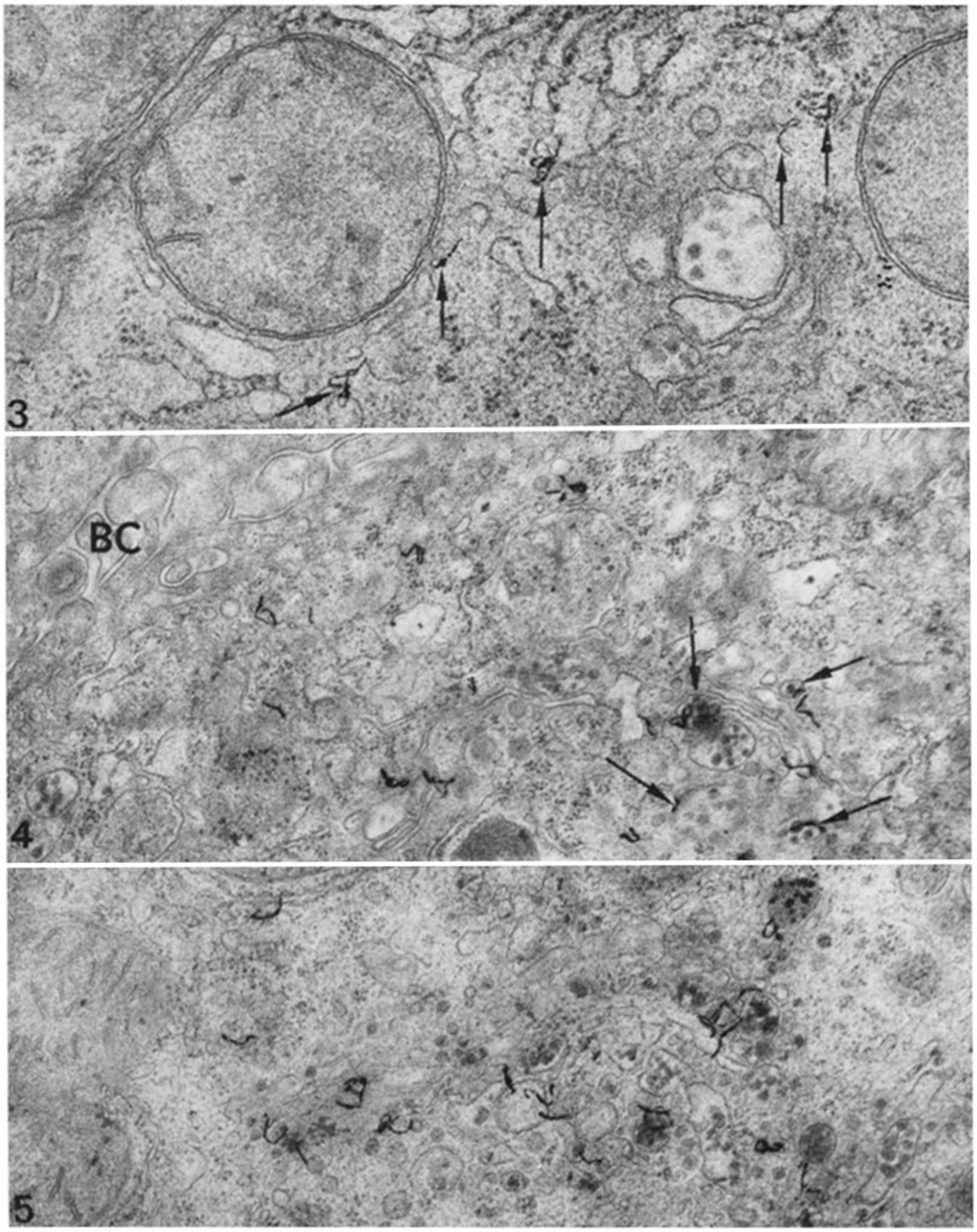
The results of this study are in agreement with the general concepts of synthesis and transport of exportable proteins in exocrine cells. Direct evidence is presented for the participation of the Golgi apparatus in the intracellular migration of albumin. In agreement with previous findings, albumin was found to be synthesized preferentially on bound ribosomes rather than on free ribosomes[2] (15, 39–41). The findings support the idea that albumin, like other exportable proteins, is transferred to the cisternae of the rough ER after its synthesis on the ribosomes and is subsequently channeled into that portion of the smooth ER which is connected with the rough ER and surrounds the Golgi region, and to the Golgi apparatus proper. Such an interpretation is in line with the autoradiographic findings, which indicate that labeled proteins first appear in the rough ER and later are concentrated in the smooth ER, Golgi cisternae, and Golgi vacuoles. Taken as a group, the plasma proteins make up the bulk of proteins synthesized by the liver (5, 42, 43). From experiments on the distribution of TCA-precipitated label, it can be calculated that the microsomal fraction, within 30 min after administration,

Figs. 3–5 are electron microscopic radioautographs showing portions of hepatic parenchymal cells from tissues fixed in OsO_4 and embedded in Epon. The emulsions on the thin sections were exposed for 6 months, and the time of development was 90 sec. All sections were stained with lead citrate and uranyl acetate.

FIGURE 3 5 min after the injection of leucine-^{3}H. Grains (indicated by arrows) are present over, or in close vicinity of, endoplasmic reticulum located near an area with liposome-containing Golgi vacuoles. × 64,000.

FIGURE 4 12 min after the injection of leucine-^{3}H. The majority of the grains are present over the Golgi apparatus [both liposome-containing vacuoles (arrows) and smooth-surfaced cisternae]. Other grains are seen over an area of apparent endoplasmic reticulum in the vicinity of the bile capillary (*BC*). × 28,000.

FIGURE 5 20 min after the injection of leucine-^{3}H. Virtually all the grains are located over the different vesicular and vacuolar structures of the Golgi apparatus. × 32,000.


3
4
BC
5

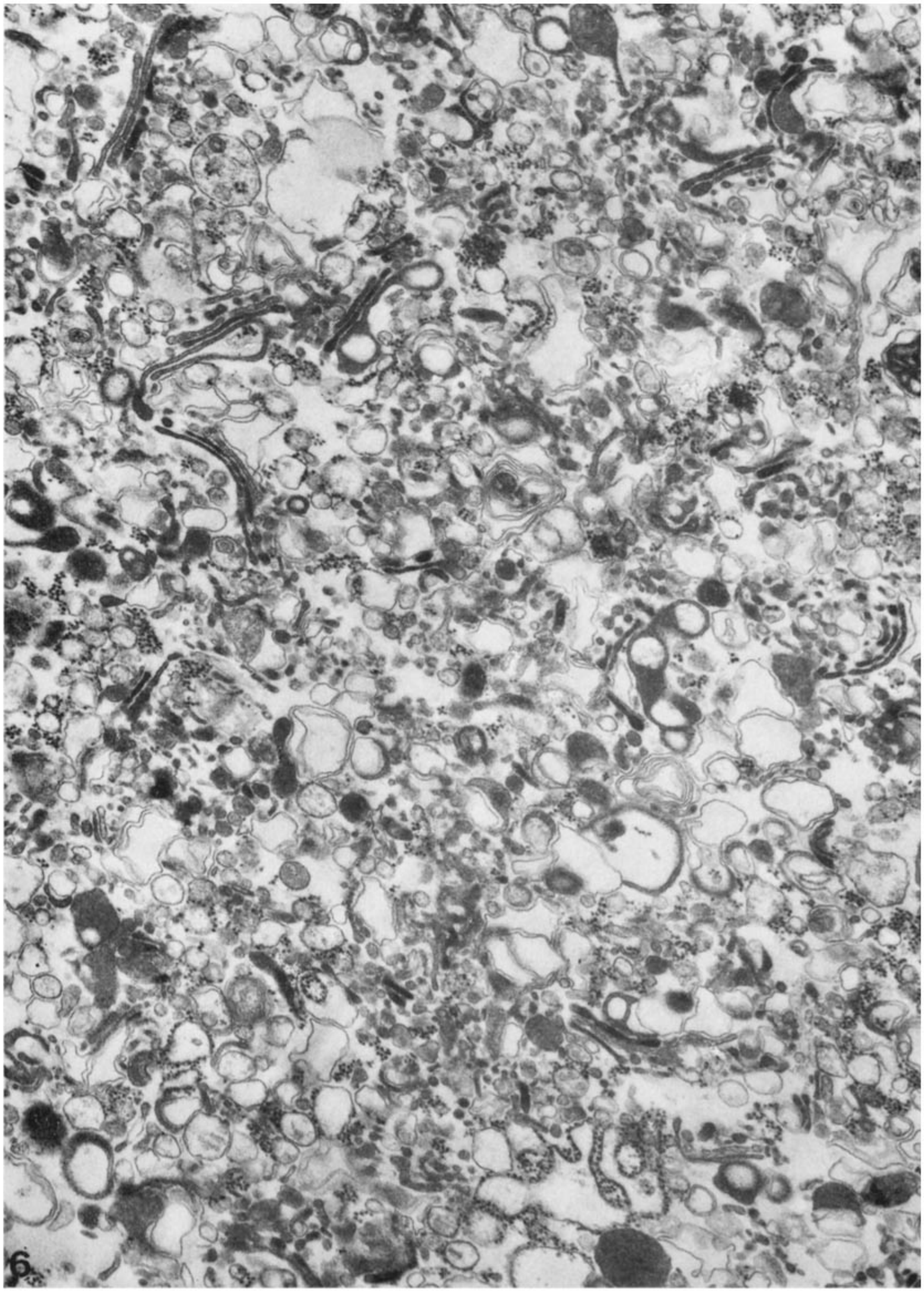


FIGURE 6 Representative area of an isolated Golgi pellet. Although there is a dominance of the different components of the Golgi apparatus, other organelles, such as apparent lysosomes and more or less fragmented portions of the rough endoplasmic reticulum, are occasionally present. Glutaraldehyde—OsO_4; Epon; lead citrate and uranyl acetate. × 15,000.

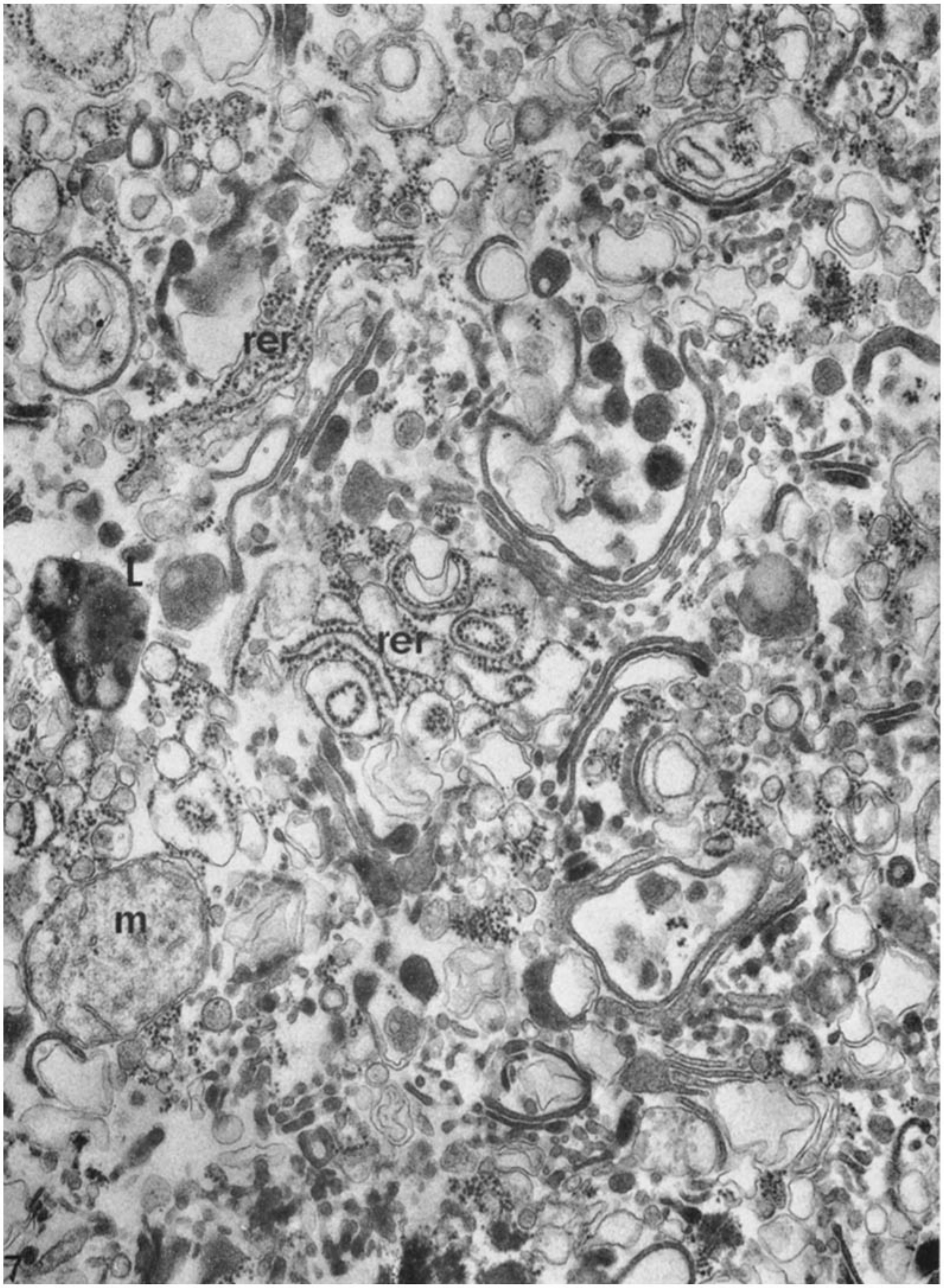


FIGURE 7 A portion of the Golgi pellet shown at higher magnification than in the previous picture. Golgi cisternae (filled with electron-opaque material) are often arranged in parallel rows and are closely associated with vesicles also containing an electron-opaque substance. Some of these vesicles seem to be in the process of budding out from the cisternae. Other organelles present in this field are a mitochondrion (*m*), a dense body (lysosome) (*L*), and meandering tubular areas of rough-surfaced endoplasmic reticulum (*rer*). Preparation as in Fig. 6. × 35,000.

accounts for ~50% of the amount of radioactivity recovered from a total tissue homogenate of liver[2]. Furthermore, about half of the total radioactivity in the microsomal membranes originated from vesicular content. Among the intravesicular proteins, albumin was found to be a predominant component. Although it is recognized that the radioautographic technique does not distinguish between exportable and nonexportable proteins, the distribution of grains should constitute a significant reflection of secretory proteins and also albumin, when put in relation to the biochemical data.

Many of the Golgi vacuoles contained liposomes. These bodies may represent very low-density lipoproteins (44, 45). As described by Jones et al., the vacuoles containing liposomes appear to move toward the plasma membrane and release their contents to the sinusoids (45). Although grains were not observed over specific structures in the sinusoidal regions, it is reasonable to assume that the labeled proteins follow the liposomes to the sinusoids (46). The studies by Ashley and Peters appear to support this notion (9). Were this so, the proteins would be discharged into the blood stream in a manner similar to the way that digestive pancreatic enzymes in zymogen granules are released to the extracellular space, viz. by membrane fusion (6).

As seen from Fig. 2, the curves for smooth microsomes and the Golgi fraction parallel one another at the first interval. The explanation for this and for the "overshooting" activity at maximal level in the Golgi fraction cannot be stated with certainty. The early appearance of labeled albumin in the Golgi fraction may, of course, be explained by contamination with microsomes, although the enzymic and morphological data do not favor such an explanation. Another possibility could be a parallel and simultaneous secretion of newly synthesized albumin from the rough ER to both smooth ER and the Golgi apparatus. The data in Table III seem to support such an interpretation, since only a small amount of the total labeled albumin eventually appeared in the Golgi fraction. However, such a conclusion is only valid in the case of an over-all high recovery of Golgi elements. At present, reliable calculation of the total amount of Golgi membranes in the liver cell cannot be made. On the other hand, the results of the radioautographic studies appear to favor the hypothesis that the smooth ER leads into the Golgi apparatus. It is not possible on the basis of available data to make a definite decision as to whether or not some of the albumin is directly transferred to the Golgi complex without first traveling through the smooth ER.

As to the "overshooting" specific activities of labeled albumin in the Golgi fraction, this may indicate that albumin of the rough ER occurs in two compartments—one exhibiting a higher rate of leucine incorporation than the other. If albumin from the former compartment was preferentially transferred to the Golgi zone, this could explain the difference in specific activities. Alternatively, it is possible that not all parts of the rough-surfaced ER participate in the specific synthesis of albumin at a given moment. If so, delivery of nascent albumin to the Golgi apparatus would occur only from those parts of the ER which were synthesizing albumin at that particular time.

The aid of Dr. Ronald R. Wagner in the measurements of glucosamine transferase is gratefully acknowledged. The authors also wish to thank Mrs. Pia Englund and Mrs. Britt-Marie Åkerman for their skillful technical assistance.

This work was supported by grants from the Swedish Medical Research Council and from Karolinska Institutet.

Received for publication 25 February 1970, and in revised form 1 June 1970.

REFERENCES

1. Siekevitz, P. 1963. *Annu. Rev. Physiol.* **25:**15.
2. Campbell, P. N., and G. R. Lawford. 1967. *In* Structure and Function of the Endoplasmic Reticulum in Animal Cells. P. N. Campbell and F. C. Gran, editors. Universitetsforlaget, Oslo. 57.
3. Straub, P. W. 1963. *J. Clin. Invest.* **42:**130.
4. Marsh, J. B., and A. F. Whereat. 1959. *J. Biol. Chem.* **234:**3196.
5. Peters, T., Jr. 1962. *J. Biol. Chem.* **237:**1181, 1186.
6. Caro, L. G., and G. E. Palade. 1964. *J. Cell Biol.* **20:**473.
7. Jamieson, J. D., and G. E. Palade. 1967. *J. Cell Biol.* **34:**577, 597.
8. Jamieson, J. D., and G. E. Palade. 1968. *J. Cell Biol.* **39:**580, 589.
9. Ashley, C. A., and T. Peters, Jr. 1969. *J. Cell Biol.* **43:**237.
10. Glaumann, H., and J. L. E. Ericsson. 1970. International Symposium on Drug Induced Metabolic Adaptations, Turku. 8.

11. DALLNER, G. 1963. *Acta Pathol. Microbiol. Scand. Suppl.* 166.
12. GLAUMANN, H., and G. DALLNER. 1968. *J. Lipid Res.* **9**:720.
13. MORRÉ, J. D., L. M. MERLIN, and T. W. KEENAN. 1969. *Biochem. Biophys. Res. Commun.* **37**:813.
14. LOEB, J. N., R. R. HOWELL, and G. M. Tomkins. 1967. *J. Biol. Chem.* **242**:2069.
15. REDMAN, C. M. 1968. *Biochem. Biophys. Res. Commun.* **31**:845.
16. PETERMANN, M. L., and A. PAVLOVEC. 1961. *J. Biol. Chem.* **236**:3235.
17. DALLNER, G., P. SIEKEVITZ, and G. E. PALADE. 1966. *J. Cell Biol.* **30**:73, 97.
18. DALLNER, G., and L. ERNSTER. 1968. *J. Histochem. Cytochem.* **16**:611.
19. CAMPBELL, P. N., O. GREENGARD, and B. A. KERNOT. 1960. *Biochem. J.* **74**:107.
20. KURIYAMA, Y., T. OMURA, P. SIEKEVITZ, and G. E. PALADE. 1969. *J. Biol. Chem.* **244**:2017.
21. LEVINE, S. 1954. *Arch. Biochem. Biophys.* **50**:515.
22. IWATA, T., H. IWATA, and J. F. HOLLAND. 1968. *Clin. Chem.* **14**:22.
23. BEATTIE, D. S., and R. E. BASFORD. 1966. *J. Biol. Chem.* **241**:1419.
24. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL. 1951. *J. Biol. Chem.* **193**: 265.
25. GLAUMANN, H., and G. DALLNER. 1970. *J. Cell Biol.* **47**:34.
26. BOWERS, E. W., J. T. FINKENSTEDT, and C. DEDUVE. 1967. *J. Cell Biol.* **32**:325.
27. WAGNER, R. R., and M. A. CYNKIN. 1969. *Biochem. Biophys. Res. Commun.* **35**:139.
28. CARO, L. G., and R. P. VAN TUBERGEN. 1962. *J. Cell Biol.* **15**:173.
29. MARSH, J. B., and D. L. DRABKIN. 1958. *J. Biol. Chem.* **230**:1073.
30. FLEISCHER, B., S. FLEISCHER, and H. OZAWA. 1969. *J. Cell Biol.* **43**:59.
31. ERNSTER, L., and H. LÖW. 1955. *Exp. Cell Res.* **3**(Suppl.):133.
32. COLEMAN, R., R. H. MICHELL, J. B. FINEAN, and J. N. HAWTHORNE. 1967. *Biochim. Biophys. Acta.* **135**:573.
33. COHN, Z. A., and M. E. FEDORKO. 1969. *In* Lysosomes in Biology and Pathology, J. T. Dingle and H. B. Fell, editors. North Holland Publishing Co., Amsterdam. **1**:43.
34. WAGNER, R. R. 1969. Glycoprotein Biosynthesis: The *in vitro* transfer of glycosyl groups to protein in subcellular fractions of rat liver. Doctoral Thesis. Tufts University, Medford, Mass.
35. ERICSSON, J. L. E. 1969. *In* The Biological Basis of Medicine. E. E. Bittar and N. Bittar, editors. Academic Press Inc., New York. **5**:143.
36. MOLNAR, J., M. TETAS, and H. CHAO. 1969. *Biochem. Biophys. Res. Commun.* **37**:684.
37. DALLNER, G., A. BERGSTRAND, and R. NILSSON. 1968. *J. Cell Biol.* **18**:503.
38. GLAUMANN, H., and S. JAKOBSSON. 1970. Proceedings of the Fourth International Congress on Pharmacology, Basel. **4**:82.
39. GANOZA, M. C., C. A. WILLIAMS, and F. LIPPMANN. 1965. *Proc. Nat. Acad. Sci. U.S.A.* **53**:619.
40. REDMAN. C. M. 1969. *J. Biol. Chem.* **144**:4308.
41. TAKAGI, M., T. TANAKA, and K. OGATA. 1969. *J. Biochem.* **65**:651.
42. GREEN, M., and L. L. MILLER. 1960. *J. Biol. Chem.* **235**:3202.
43. RYOO, H., and H. TARVER. 1968. *Proc. Soc. Exp. Biol. Med.* **128**:760.
44. HAMILTON, R. L., D. M. REGEN, M. E. GRAY, and V. S. LEQUIRE. 1967. *Lab. Invest.* **16**:305.
45. JONES, A. L., N. B. RUDERMAN, and M. G. HERRERA. 1967. *J. Lipid Res.* **8**:429.
46. BRUNI, C., and K. R. PORTER. 1965. *Amer. J. Pathol.* **46**:691.
47. CHAUVEAU, J., Y. MOULÉ, C. ROUILLER, and J. SCHNEBELLI. 1962. *J. Cell. Biol* **12**:17.
48. PITOT, H. C. 1964. *Perspect. Biol. Med.* **8**:50.
49. GARDNER, J. A. A., and M. B. HOAGLAND. 1968. *J. Biol. Chem.* **243**:10.