INTRACELLULAR TRANSPORT OF SECRETORY PROTEINS IN THE PANCREATIC EXOCRINE CELL

I. Role of the Peripheral Elements of the Golgi Complex

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ABSTRACT

It has been established by electron microscopic radioautography of guinea pig pancreatic exocrine cells (Caro and Palade, 1964) that secretory proteins are transported from the elements of the rough-surfaced endoplasmic reticulum (ER) to condensing vacuoles of the Golgi complex possibly via small vesicles located in the periphery of the complex. To define more clearly the role of these vesicles in the intracellular transport of secretory proteins, we have investigated the secretory cycle of the guinea pig pancreas by cell fractionation procedures applied to pancreatic slices incubated in vitro. Such slices remain viable for 3 hr and incur minimal structural damage in this time. Their secretory proteins can be labeled with radioactive amino acids in short, well defined pulses which, followed by cell fractionation, makes possible a kinetic analysis of transport. To determine the kinetics of transport, we pulse-labeled sets of slices for 3 min with leucine-^{14}C and incubated them for further +7, +17, and +57 min in chase medium. At each time, smooth microsomes (=peripheral elements of the Golgi complex) and rough microsomes (= elements of the rough ER) were isolated from the slices by density gradient centrifugation of the total microsomal fraction. Labeled proteins appeared initially (end of pulse) in the rough microsomes and were subsequently transferred during incubation in chase medium to the smooth microsomes, reaching a maximal concentration in this fraction after +7 min chase incubation. Later, labeled proteins left the smooth microsomes to appear in the zymogen granule fraction. These data provide direct evidence that secretory proteins are transported from the cisternae of the rough ER to condensing vacuoles via the small vesicles of the Golgi complex.

INTRODUCTION

The pancreatic exocrine cell of mammals synthesizes large quantities of secretory proteins, i.e., enzymes and enzyme precursors (zymogens), stores them temporarily within secretion granules, and discharges them upon proper stimulation into the glandular lumina. For these reasons, it represents a favorable and often used cell type for studying initial events involved in the synthesis of secretory proteins, as well as subsequent events connected with their segregation and preparation for eventual discharge.

At present, the essential features of the secretory cycle of the exocrine pancreas are clearly defined (Palade, Siekevitz, and Caro, 1962) and can be divided into four steps: 1. The incorporation of amino acids into the well characterized digestive enzymes (e.g., amylase) or zymogens (e.g., α-chymotrypsinogen); this step is known to take place on ribosomes attached to the membrane of

the rough-surfaced endoplasmic reticulum (ER)' (Siekevitz and Palade, 1960; Redman, Siekevitz and Palade, 1966). 2. Transfer and segregation of secretory proteins, immediately upon completion of their synthesis, into the cisternae of the rough-surfaced endoplasmic reticulum (Redman et al., 1966; see also Redman and Sabatini, 1966). 3. Intracellular transport of these proteins to the Golgi complex where they are packaged into zymogen granules (Caro and Palade, 1964). 4. Migration of zymogen granules to the cell apex and, finally, discharge of their content into the glandular lumina (Palade, 1959) from which they will eventually reach the intestine.

Steps 1, 2, and 4 have been satisfactorily documented in the literature. Less well understood is step 3, i.e., the transfer of secretory proteins to the Golgi complex and the formation of zymogen granules therein. The radioautographic studies of Caro and Palade (1964) have provided convincing evidence that secretory proteins are transported to, and concentrated within, the condensing vacuoles of the Golgi complex which, in the process, become zymogen granules. But the route followed by these proteins from the rough ER to the condensing vacuoles remained uncertain. The work showed that these proteins reach the Golgi complex less than 10 min after their synthesis, and suggested that they are associated with the masses of small, smooth-surfaced vesicles characteristically located at the periphery of the complex. However, the resolving power of the radioautographic technique was not sufficient to decide whether the labeled proteins were located within these vesicles or in the surrounding cytoplasmic matrix. This point requires clarification since it has been postulated, on the basis of cell fractionation data, that secretory proteins are transported from microsomes (mostly the rough ER in situ) through the cell sap (cytoplasmic matrix in situ) to the zymogen granules (Redman and Hokin, 1959; Morris and Dickman, 1960) or the acinar lumen (Redman and Hokin, 1959).

To clarify the role of the Golgi elements in the intracellular transport of secretory proteins, we have reinvestigated the secretory cycle of the guinea pig pancreas using a system of pancreatic slices incubated in vitro.[1] With this system, it is possible to obtain a well defined pulse labeling of secretory proteins of sufficiently short duration (2–3 min) to allow us to analyze the relatively rapid (<10 min) phase of intracellular transport with which we are concerned. In the whole animal, labeling pulses shorter than ~ 15 min cannot be obtained (Caro and Palade, 1964). In addition, we have isolated from incubated slices a fraction of rough microsomes (representing the rough ER), and one of smooth microsomes (representing primarily the peripheral elements of the Golgi complex) by density gradient centrifugation, and have determined the kinetics of transport of labeled proteins from one fraction to the other.

In this paper, the characteristics of the pancreatic slice system will be described and data presented to demonstrate directly the role of the Golgi complex in the intracellular transport of secretory proteins. In the succeeding paper, the subsequent fate of secretory proteins during the formation of zymogen granules will be documented by combined cell fractionation and electron microscopic radioautographic studies.

METHODS

ANIMALS: Male albino guinea pigs ~ 3 months old and 500 g in weight, were fasted for 24–27 hr, were stunned by a blow to the head, and were bled by cardiac section. The pancreatic glands were removed and placed in iced incubation medium.

SLICING PROCEDURE: After mesentery, fat, and damaged tissue was trimmed away, each pancreas was cut into pieces $\sim 5 \times 7$ mm with scissors, and each piece was sectioned into two to three slices $\sim 0.5 \times 7 \times 5$ mm in size and ~ 50 mg wet weight with a Stadie-Riggs tissue-slicing blade. All operations were performed at 4°. Enough slices (usually ~ 30) for cell fractionation at three time points can be obtained from the pancreas of one animal though for more extensive experiments, slices were pooled from the glands of two animals.

INCUBATION PROCEDURE: The incubation medium was a Krebs-Ringer bicarbonate solution (Krebs, 1950) equilibrated with 95% O_2, 5% CO_2 to a final pH of 7.6 and containing 14 mM glucose. In most experiments, it was supplemented with L-amino acids at concentrations specified for Eagle's tissue culture medium (Eagle, 1959), except that, for tracer experiments with L-leucine-^{14}C or L-leucine-^{3}H, unlabeled leucine was omitted from the amino acid supplement.

PULSE LABELING: For cell fractionation experiments, pulse labeling was performed as follows: 50-ml flasks containing 7 ml of incubation medium, to which uniformly labeled L-leucine-^{14}C was added to a concentration of 1 μC/ml (4.5 μM), were loaded

[1] As used in this paper, in vitro refers to incubated pancreatic slices while in vivo refers to the pancreas in situ, i.e., in the whole animal.

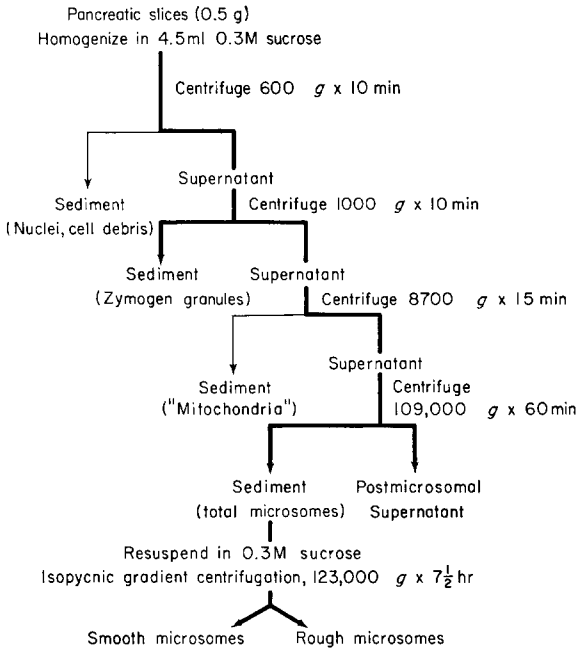


FIGURE 1 Cell fractionation scheme applied to guinea pig pancreatic slices. Centrifugal forces are average values in the middle of the tube.

at 0° with 7 slices,[2] gassed, and kept on ice for 10 min. The flasks were then incubated in a water bath at 37° with agitation at 100 cycle/min. After 3 min, the contents of each flask were poured into gauze-lined funnels and washed with warm aerated chase medium which contained either 3.5 or 0.4 mM (780× or 90× excess) of L-leucine-^{12}C. One set of slices was placed in iced 0.3 M sucrose and immediately homogenized; the remaining sets of slices were placed in 20 ml of warm chase medium in a 125-ml flask, gassed, and incubated for further times at 37° with shaking. At the end of each period, the slices were again washed, placed in iced 0.3 M sucrose, and homogenized.

CELL FRACTIONATION SCHEME: Groups of seven slices (~0.5 g wet weight) were homogenized and fractionated according to a standardized scheme:

All operations were carried out at 4°.

1. Slices were minced finely with scissors and 0.3 M sucrose added to give a final tissue concentration of 1/10, w/v.

2. Homogenization was completed by three passes in a Brendler type (Brendler, 1951) tissue grinder driven by a motor at 3000 rpm.

3. Differential centrifugation: The homogenate was fractionated using the differential centrifugation scheme shown in Fig. 1. Centrifugal forces given are average values at the center of the tube. After removal of nuclei and cell debris, zymogen granules were isolated from the supernatant by low speed centrifugation (Hokin, 1955). The tightly packed, white zymogen granule pellet was overlaid with a loose tan layer of mitochondria which could be removed by gently washing the surface of the pellet with a small volume of 0.3 M sucrose. The mitochondrial supernate was removed with a pipette, care being taken to avoid the loose sediment above the pellet, and centrifuged at 109,000 g × 60 min. The resulting microsomal pellet was pale pink, translucent, and firmly packed.

4. GRADIENT CENTRIFUGATION: Rough- and smooth-surfaced microsomes were isolated as follows. The common microsomal pellet was gently resuspended in 0.3 M sucrose with a teflon pestle, and 0.25 ml of the resuspension (~2 mg protein) was layered over a 4.5-ml linear sucrose density gradient (Britten and Roberts, 1960) ranging from 1.04 to 2.0 M sucrose (density at 4°, 1.14–1.27 g/cc). The gradients were centrifuged at 123,000 g (average) for 7½ hr at 4° in a Spinco model L2 centrifuge (SW 39L rotor). At the end of the run, the three major bands which formed in the gradient were collected with a J-shaped needle attached to a syringe. Each sample was diluted to 0.3 M sucrose and centrifuged at 109,000 g for 90 min in a Spinco No. 40.3 rotor. The ensuing pellets were fixed in situ for electron microscopy, or resuspended in water for biochemical assays.

Radioactivity and Chemical Assays

1. RADIOACTIVITY ASSAYS: The resuspended particulate fractions and supernate fractions were

[2] For experiments in which only total incorporation of label into protein was determined, two slices per flask sufficed.

precipitated at 4° with trichloroacetic acid (TCA), 10% final concentration, left on ice for 2 hr, and centrifuged. Aliquots of the cold TCA-soluble material were counted directly in a *p*-dioxane-base liquid scintillation fluid (Bray, 1960).

TCA precipitates were washed three times with cold 5% TCA, dissolved in 0.2 ml 88% formic acid, and transferred to scintillation-counting vials. Extraction of lipids with organic solvents and of nucleic acids with TCA at 90° (Schneider, 1945) prior to counting TCA precipitates was not routinely performed as it was found that this procedure removes less than 5% of the TCA-insoluble radioactivity (incorporated as L-leucine-^{14}C uniformly labeled or as L-leucine-4,5-^{3}H). All counting rates reported have been corrected for quenching and background. Unquenched ^{14}C samples counted with an efficiency of 85%.

2. CHEMICAL ASSAYS: Proteins were determined by the method of Lowry et al. (1951) on TCA precipitates dissolved in 1 N NaOH. Crystalline bovine serum albumin was used as standard.

The RNA content of TCA precipitates was measured by the orcinol reaction (Mejbaum, 1939) on hot TCA-soluble extracts (5% TCA, 90° for 20 min); purified yeast RNA was used as a standard.

Phospholipids were extracted from TCA precipitates by the Folch procedure (Folch, 1952) and the phospholipid-phosphorus estimated on H_2SO_4 digests (Bartlett, 1959).

Microscopy

1. FIXATION AND EMBEDMENT: Pancreatic slices were immersed in cold fixative, cut into blocks about 0.5 mm a side, and fixed for 3–4 hr at 0° in 1% OsO_4 buffered at pH 7.4 with 0.1 M potassium phosphate. After washing with several changes of 0.17 M acetate-veronal buffer, pH 6.0, the blocks were stained for 2 hr at room temperature with 0.5% uranyl acetate in the same buffer (Ryter and Kellenberger, 1958; Farquhar and Palade, 1965). For light and electron microscopic radioautography, this step was omitted. Tissue blocks were dehydrated in increasing concentrations of ethanol followed by propylene oxide and embedded in Epon 812 (Luft, 1961).

Pellets of cell fractions were fixed in situ in cellulose nitrate centrifuge tubes for 18 hr at 0° with 2% OsO_4, buffered at pH 7.4 with 0.1 M potassium phosphate. The fixed pellets were cut into strips so oriented that the direction of centrifugation could be determined after embedment. Further processing was the same as for tissue. Prior to sectioning, the pellets were remounted so that the plane of sectioning would exactly parallel the axis of sedimentation. Thus, the full depth of the pellet was included in each section.

2. SECTIONING AND STAINING: All sections were cut with a diamond knife on a Porter-Blum microtome. Tissue sections were mounted on naked 300-mesh copper grids, and pellet sections on Formvar-coated bar-grids with the axis of sedimentation parallel, or nearly parallel, to the bars. The use of bar-grids permits uninterrupted viewing from the top to the bottom of the pellet. Sections were doubly stained with alcoholic-uranyl acetate and basic lead citrate (Venable and Coggeshall, 1965).

3. MICROSCOPY: The preparations were examined and micrographed in a Siemens Elmiskop I operated at 80 kv and equipped with a 200-μ condenser aperture and a 50-μ objective aperture.

Materials

All chemicals were reagent grade. L-amino acids were purchased from Sigma Chemical Company, St. Louis. Uniformly labeled L-leucine-^{14}C (specific activity 240 mc/mmole) was obtained from the New England Nuclear Corporation, Boston.

RESULTS

Characteristics of Slice System

Since, to our knowledge, surviving guinea pig pancreatic slices have not been used before to study either synthesis or intracellular transport of secretory proteins, it was necessary at the outset of this study to find out whether the system is able to incorporate radioactive amino acids from the medium into proteins and if the slice proteins can be pulse labeled with the tracer. In addition, it was necessary to determine the extent of structural damage—if any—incurred by the incubated tissue slices, because a satisfactory answer to the problem under consideration depends entirely on retention of the structural integrity and precise intracellular relationships of the subcellular components of the exocrine cells.

KINETICS OF INCORPORATION OF L-LEUCINE-^{14}C INTO TOTAL SLICE PROTEINS: To determine the rate of incorporation of leucine-^{14}C into their proteins, we incubated slices obtained from a single pancreas in parallel in medium containing tracer and in the same medium supplemented with amino acids minus leucine-^{12}C. In the nonsupplemented medium (Fig. 2), incorporation of leucine into protein proceeded actively for 1 hr, presumably at the expense of the endogenous supply of other amino acids in the tissue. After 1 hr, incorporation gradually decreased and after 2 hr it stopped. In the presence of a complete amino acid supplementation, incorporation of the label into protein proceeded at a linear rate for 3

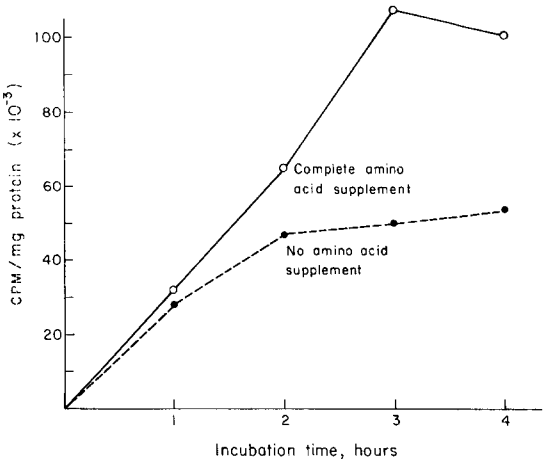


FIGURE 2 Kinetics of L-leucine-^{14}C (uniformly labeled; 0.2 μc/ml; 0.82 μM) incorporation into total slice proteins. Solid circles: incubation in Krebs-Ringer bicarbonate medium without amino acid supplement. Open circles: incubation in Krebs-Ringer bicarbonate medium with complete amino acid supplement (minus L-leucine-^{12}C). Each time point is based on radioactivity and protein determinations from two pancreatic slices ($\sim$ 100 mg wet weight of tissue).

hr; between 3 and 4 hr, net incorporation usually stopped for unknown reasons. During the 1st hr, the rates of incorporation in both nonsupplemented and supplemented media were about the same; presumably thereafter, the endogenous supply of amino acids became rate-limiting in the nonsupplemented medium, resulting in a gradual decrease in incorporation rate. Hokin (1951 *a*, *b*) has also reported that net synthesis of amylase in pigeon pancreatic slices is dependent on addition of amino acids to the incubation medium. As a result of these findings, the majority of the experiments reported was performed in amino acid-supplemented medium.

CHARACTERISTICS OF PULSE LABELING: Investigation of the kinetics of intracellular transport of secretory proteins in the pancreatic system requires true pulse labeling of these proteins with radioactive amino acids for periods significantly shorter than those involved in their transport to the next intracellular compartment, i.e., <10 min (Caro and Palade, 1964).

To find out whether pulse labeling can be achieved and, if so, to determine its characteristics, we performed a series of experiments of the type illustrated by Fig. 3. Slices were put in tracer-containing incubation medium and kept at 0° for 10 min. During this period, the tracer diffuses into the tissue though no incorporation into proteins occurs (see Fig. 4). This period was followed by incubation for 3 min at 37°, after which the slices were washed, transferred to chase medium, and further incubated therein for times up to 30 min. The results showed (Fig. 3) that transfer to chase medium resulted in rapid washout of cold TCA-soluble radioactivity from the slices. This effect, plus the isotope dilution by excess leucine-^{12}C in the chase medium, rapidly and effectively stopped further incorporation of label as indicated by the unchanging specific radioactivity of labeled slice proteins during the following 30-min incubation. Thus, a true pulse label was obtained. Concentrations of either 3.5 or 0.4 mM leucine-^{12}C in the chase medium were equally effective in terminating further incorporation, although omission of excess leucine-^{12}C from the chase medium allowed some further incorporation of label. In separate experi-

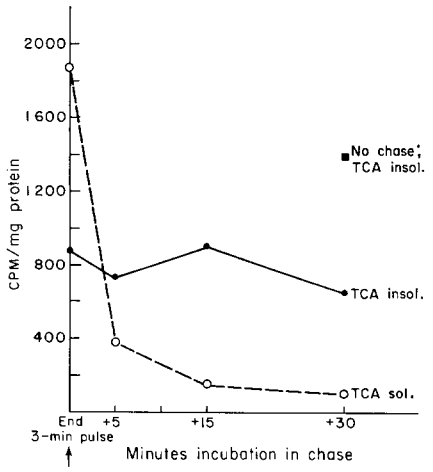


FIGURE 3 Kinetics of pulse labeling of pancreatic slice proteins. Pulse medium: Krebs-Ringer bicarbonate with amino acid supplement (minus leucine-^{12}C) and containing 0.4 μc/ml L-leucine-^{14}C (1.6 μM). Chase medium: Krebs-Ringer bicarbonate with amino acid supplement (minus leucine-^{14}C) and containing L-leucine-^{12}C (1.75 mM). Solid circles: labeled protein in slices incubated in chase medium. Open circles: cold TCA-soluble radioactivity in slices incubated in chase. Solid square: labeled protein in slices incubated post-pulse in Krebs-Ringer bicarbonate with amino acid supplement, but without added L-leucine-^{12}C. Each time point is the average of radioactivity and protein determinations on two pancreatic slices.

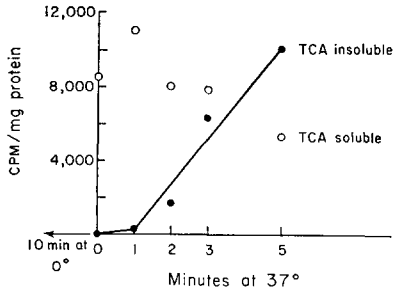


FIGURE 4 Incorporation of L-leucine-^{14}C into TCA-soluble and insoluble fractions of slices during first 5 min of incubation in pulse medium consisting of Krebs-Ringer bicarbonate with amino acid supplement (minus L-leucine-^{12}C) and containing 1.0 μc/ml (4 μM) L-leucine-^{14}C. Determinations of radioactivity and protein at each time point performed on the pooled tissue of two pancreatic slices. Solid circles: labeled protein in slices. Open circles: cold TCA-soluble radioactivity. Note that during 10 min of incubation at 0° there was no incorporation of label into proteins though label accumulated in the cold TCA-soluble fraction of the tissue.

ments not reported here, it was found that the highest concentration of leucine-^{12}C used in the chase medium did not inhibit the synthesis of proteins by the slice. In fact, a slight stimulation occurred.

The unchanging specific radioactivity of total slice proteins during post-pulse incubation indicated that no detectable turnover of label into nonprotein material occurred.

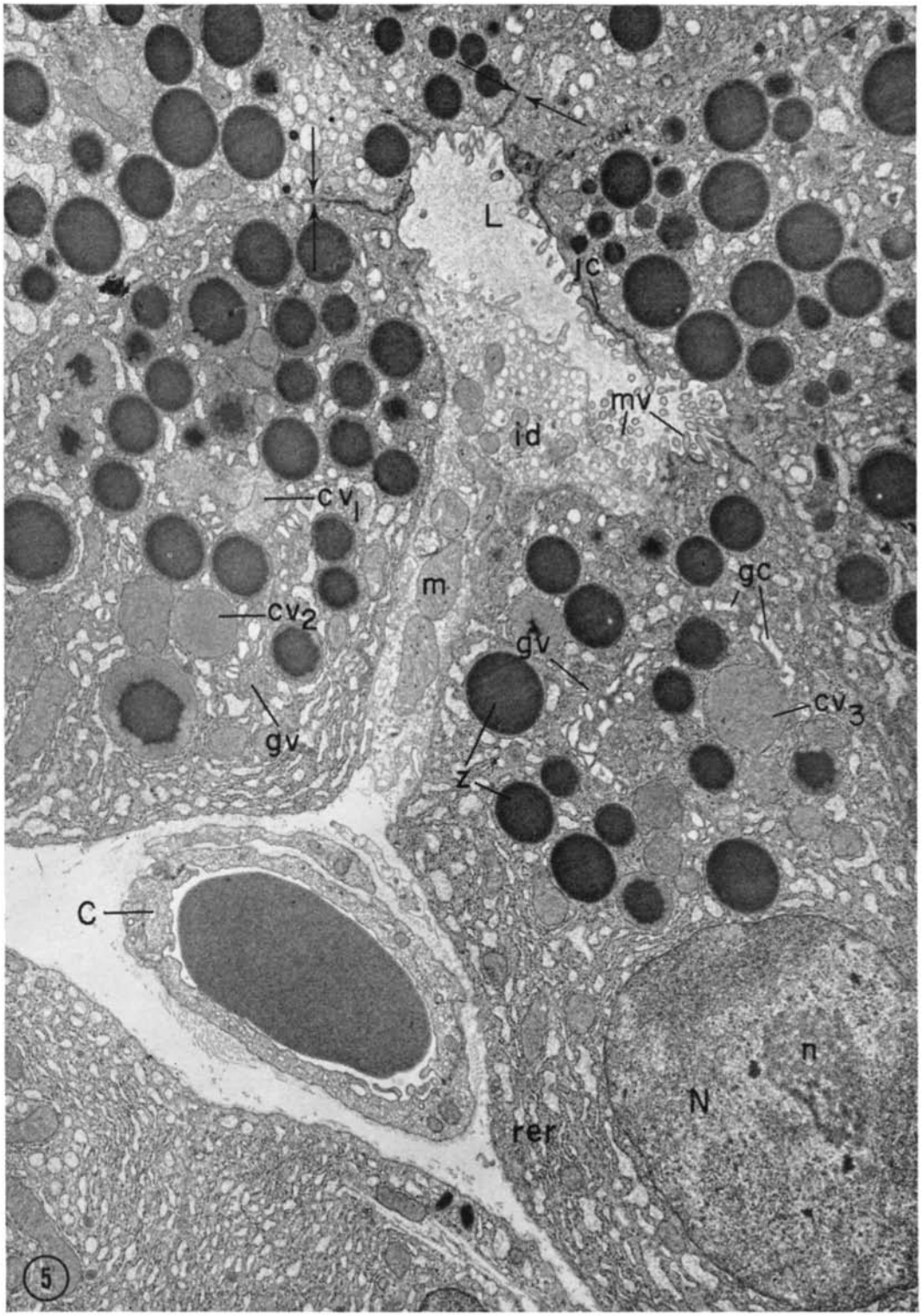
Since about 1 min is required to raise the temperature of the flask and its contents to 37°, the actual length of the pulse is 2 min, as seen in Fig. 4.

MORPHOLOGY OF INCUBATED PANCREATIC SLICES: To determine the degree of tissue and cell disorganization which occurs during preparation of slices and subsequent incubation, we examined pancreatic slices by electron microscopy before and after 3 hr of incubation at 37° in amino acid–supplemented medium.

Examination of acinar cells from unincubated slices revealed normal cytological details; only cells cut open during the preparation of slices appeared damaged. Since the in situ structure of the exocrine cell of the guinea pig pancreas is well known (see reviews by Palade, 1961; Palade, Siekevitz, and Caro, 1962), only the structure of acinar cells in incubated pancreatic slices will be described here.

The general organization of the gland, its acini, and its exocrine cells was remarkably well preserved up to 3 hr of incubation in vitro (Fig. 5). The cisternae of the rough ER appeared somewhat more distended and less regularly packed (Figs. 5, 6) than in vivo, but the rest of the cell showed little or no sign of disorganization. In particular, the elements of the Golgi complex, i.e., the peripheral swarms of small (diam. ~500 A) smooth-surfaced vesicles, the piles of smooth-surfaced cisternae, and the condensing vacuoles usually located in the center of the Golgi region, appeared unchanged in morphology and location relative to one another (Fig. 7). The same applied to the transitional elements, i.e., the part rough- and part smooth-surfaced cisternae found along the boundary between the Golgi complex and the rough ER. As in the normal, the transitional elements were characterized by small smooth-surfaced vesicles which seem either to bud from, or fuse with, their limiting membrane facing the Golgi complex. Many of the small, smooth-surfaced vesicles of the free, as well as of the "budding" variety, are covered by a finely fibrillar coat on their cytoplasmic surface comparable in appearance to that found around certain pinocytic

FIGURE 5 Low magnification electron micrograph of several pancreatic exocrine cells sectioned almost along their basal-apical axes. Slice incubated in vitro for 3 hr. The apices of the cells, provided with microvilli (*mv*), converge on an acinar lumen (*L*). Junctional complexes which join the plasma membranes of adjacent cells near the lumen are indicated by *jc*. The intercellular spaces between exocrine cells (apposed arrows) are not wider than in vivo. An intercalated duct cell (*id*) separates two exocrine cells. The main structural features of the exocrine cell are indicated: basal rough ER (*rer*); elements of the Golgi complex: piled Golgi cisternae (*gc*), peripheral vesicles (*gv*), and a series of condensing vacuoles of increasing maturity (cv_1, cv_2, cv_3); zymogen granules (*z*). *N*, nucleus; *n*, nucleolus· *C*, capillary; *m*, mitochondrion. × 12,000.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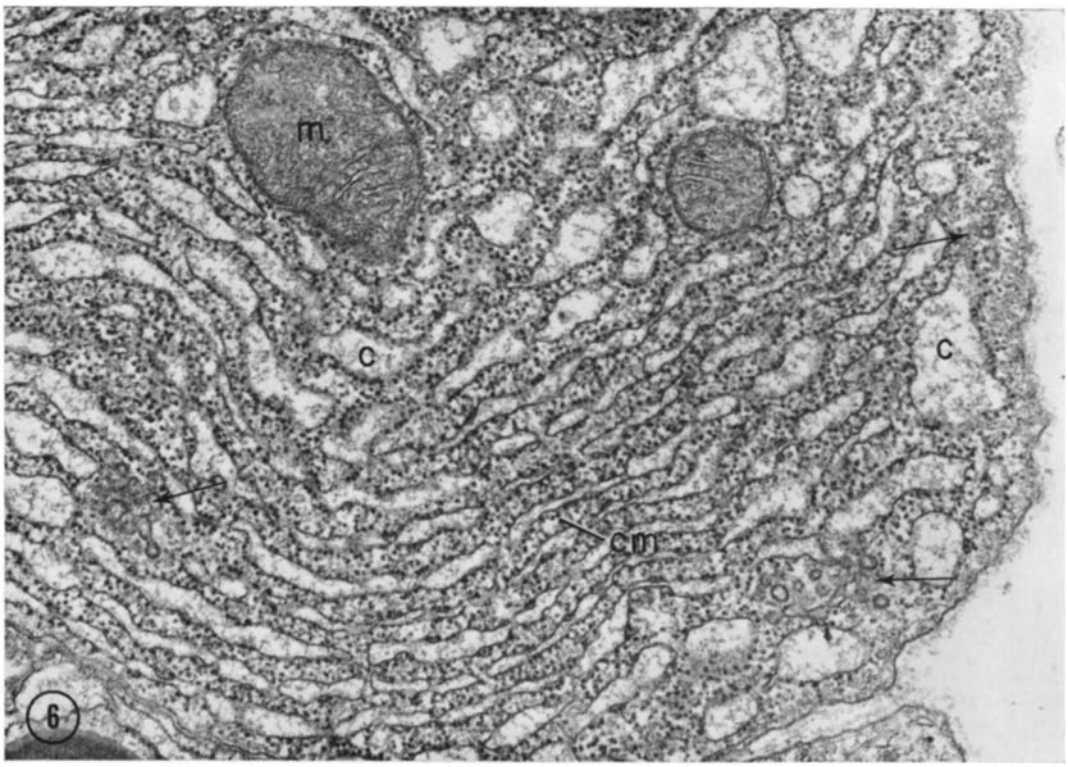
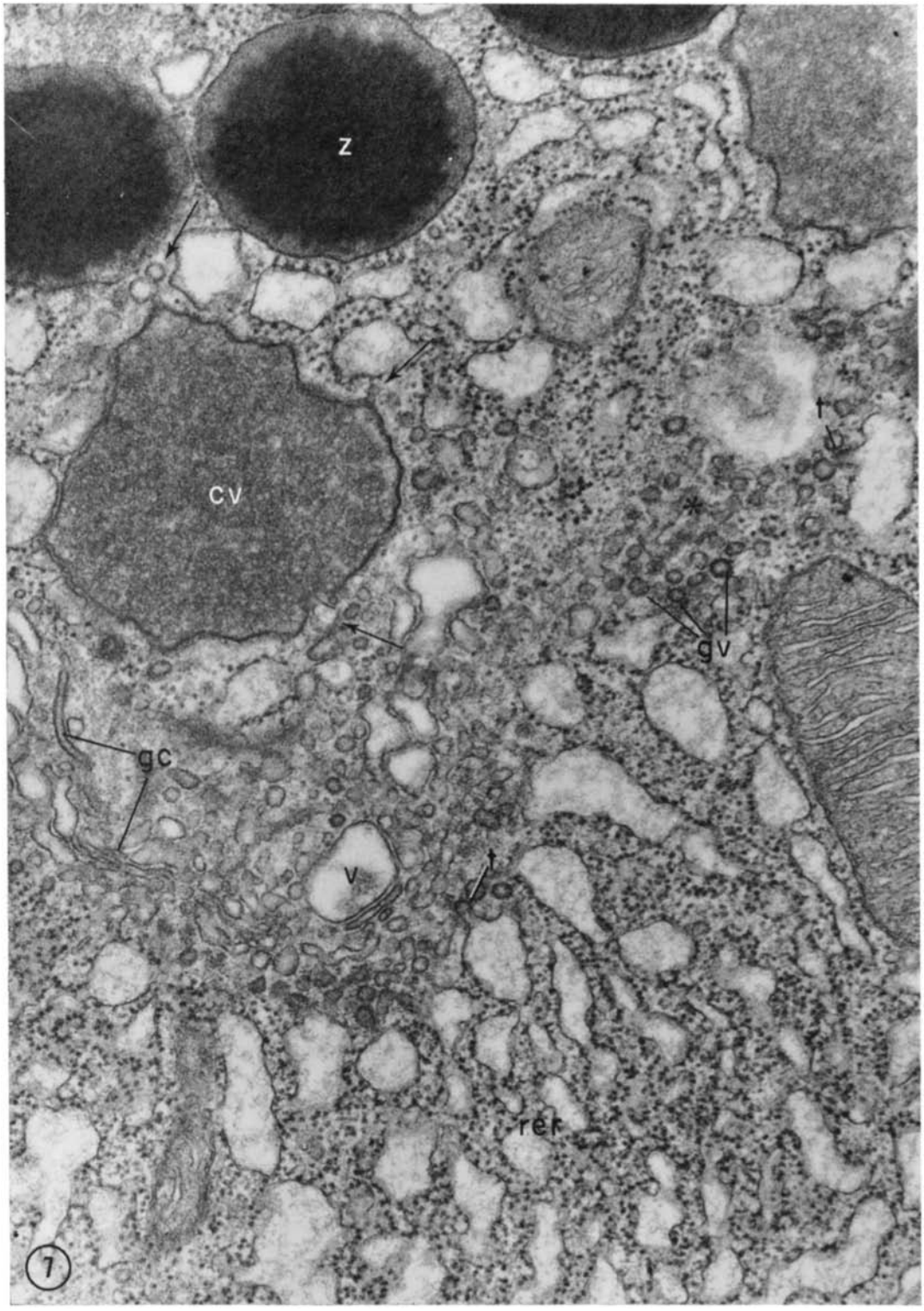


FIGURE 6 High magnification view of the basal portion of an exocrine cell from a slice incubated 3 hr, showing the stacked cisternae of the rough ER. The cisternal space (*c*) of the rough ER, bounded by membranes carrying attached ribosomes, contains fluffy, loosely packed material. Numerous "free" ribosomes are also located in the cytoplasmic matrix (*cm*). Arrows indicate collections of small, smooth-surfaced vesicles scattered among the rough ER elements located beneath the basal cell membrane. *m*, mitochondrion. × 30,000.

vesicles (Roth and Porter, 1962; Bowers, 1965). A similar coat occasionally covers sectors of the limiting membrane of condensing vacuoles. The zymogen granules in the apical region of the cells also retained their normal structure, but occasionally a less opaque zone could be distinguished at their periphery suggestive of partial swelling or extraction (Figs. 5, 8).

FIGURE 7 General view of the Golgi complex of an acinar cell from a slice incubated for 3 hr. The rough ER (*rer*) immediately adjacent to the periphery of the Golgi complex consists of part rough-, part smooth-surfaced cisternae which represent the transitional elements of the ER. The latter frequently project toward the periphery of the complex as smooth-surfaced buds (*t*). The periphery of the complex contains a large number of small, smooth-surfaced vesicles (*gv*) which occasionally appear to fuse, forming alveolate and tubelike structures (*). Smaller Golgi vacuoles (*v*) and a few piled Golgi cisternae (*gc*) occur in the same general region. Centrally located in the complex is a large condensing vacuole (*cv*) with its characteristically scalloped border and content of intermediate electron opacity. The arrows point to smooth-surfaced blebs which are often seen adjacent to or fused with the limiting unit membrane of the condensing vacuole. *z*, zymogen granule. × 44,000.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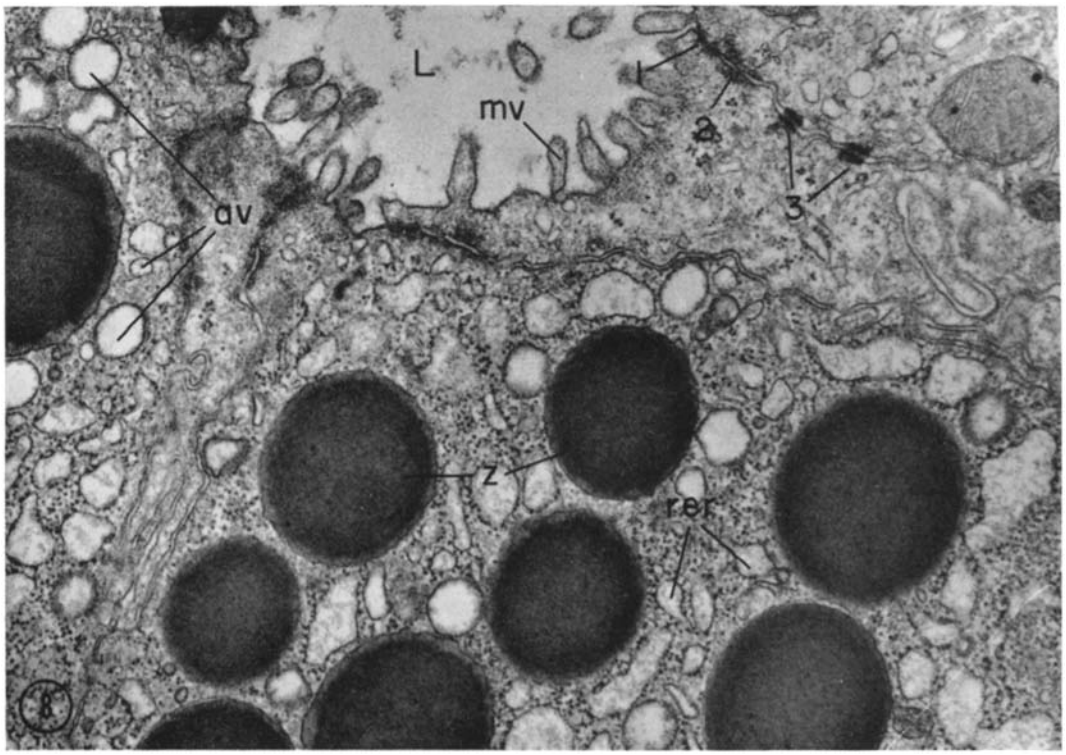


FIGURE 8 Micrograph of the apical region of an exocrine cell from a slice incubated for 3 hr. The plasma membranes of contiguous cells are held together by junctional complexes consisting of occluding zonules (*1*), adhering zonules (*2*), and adhering maculae (*3*) or desmosomes. The cytoplasm near the lumen is packed with zymogen granules (*z*) which are bounded by a unit membrane and filled with a dense, homogeneous content. Note the lighter halo at the periphery of some granules. The remaining cytoplasm is occupied by a few rough ER cisternae (*rer*) and numerous smooth-surfaced vesicles and vacuoles (*av*). *L*, acinar lumen; *mv*, microvilli. × 26,000.

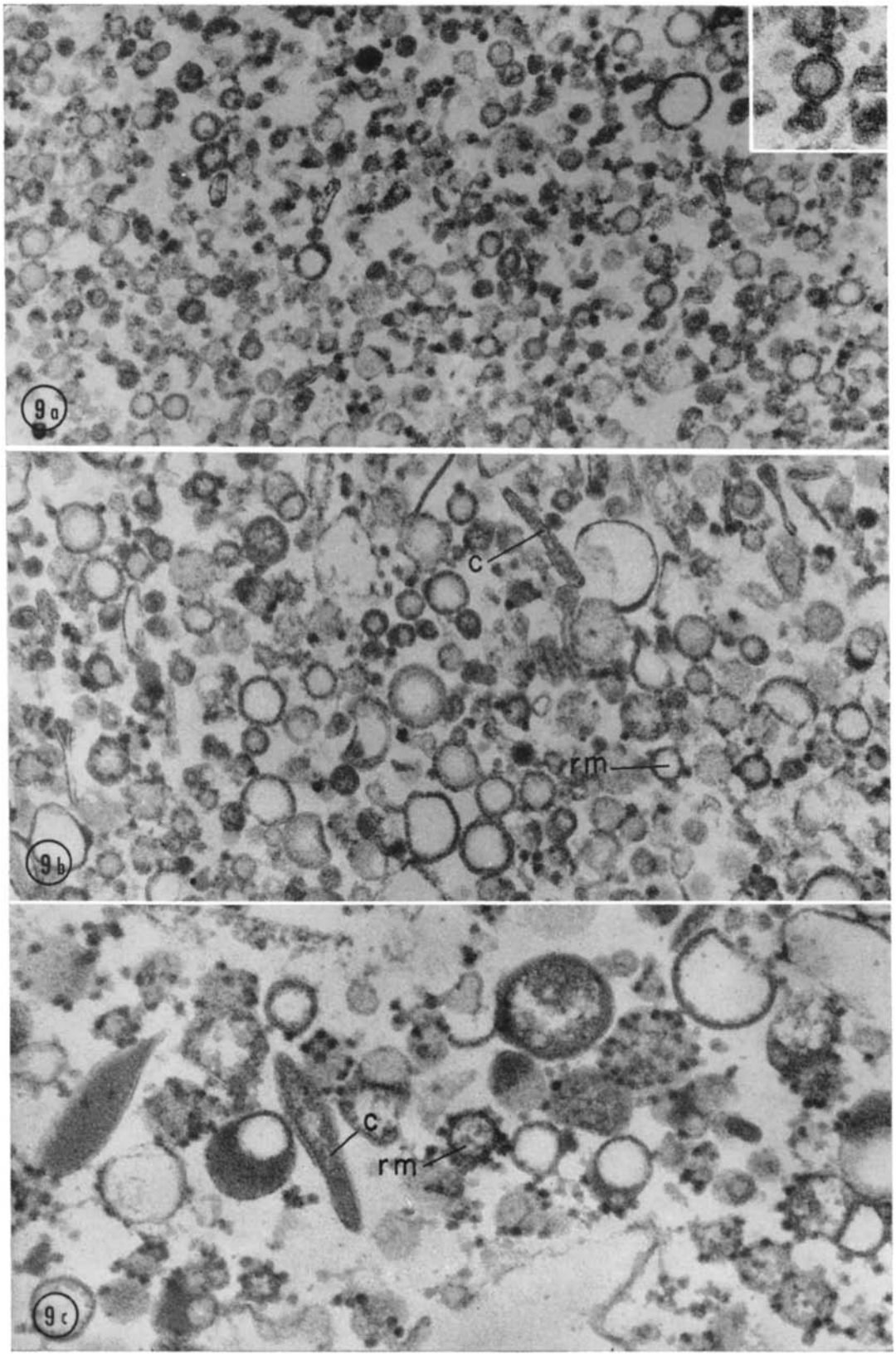
While the majority of the acinar cell population showed no damage in incubated slices, a small number of cells, usually located near cut surfaces, were affected by obvious and characteristic degenerative changes which consisted either of aggregations of densely staining material in the cytoplasmic matrix, of dissolution and loss of opacity of zymogen granules, or of accumulations of cell debris in membrane-enclosed autolytic vacuoles (see Hruban, Swift, and Wissler, 1962).

FIGURES 9 *a–c* Micrographs of the top (Fig. 9 *a*), middle (Fig. 9 *b*), and bottom (Fig. 9 *c*) thirds of the smooth microsomal pellet. × 70,000.

The upper third of the pellet (Fig. 9 *a*) consists entirely of small, smooth-surfaced vesicles bounded by a unit membrane (inset).

The middle third of the pellet (Fig. 9 *b*) contains, in addition, some larger vacuoles (~150 mμ in diameter) and flattened cisternae (*c*). A rare rough microsome (*rm*) is seen in this portion of the pellet.

Fig. 9 *c* is a micrograph taken at the extreme base of the lower third of the pellet to show the stratified layer of rough microsomal (*rm*) contaminants. The majority of this portion of the pellet is similar in appearance to Fig. 9 *b*. *c*, cisternal element.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The aggregations of dense material in the cytoplasmic matrix occurred in foci deep within regions occupied by rough endoplasmic reticulum. The adjacent cisternal membranes appeared free or almost free of attached ribosomes, which suggested that the aggregates represented products of ribosomal degradation. Masses of similar dense material were also seen at the periphery of the nucleolus.

Transport of Secretory Proteins from the Rough Endoplasmic Reticulum to the Periphery of the Golgi Complex

METHOD OF ISOLATION OF SMOOTH AND ROUGH MICROSOMES: To test the assumption that secretory proteins are transported from the rough ER to the small vesicles at the periphery of the Golgi complex, it was necessary for us to isolate relatively homogeneous fractions, representative of these two subcellular components. Since it is known (Palade and Siekevitz, 1956 *b*) that small, smooth vesicles are present in microsomal pellets, and occur in smaller numbers in post-microsomal fractions, the common microsomal fraction was used as the starting material for further subfractionation into rough and smooth microsomes. The latter are expected to consist mainly of smooth-surfaced vesicles and cisternae of the Golgi complex, since these represent the most abundant source of such elements. Smooth-surfaced elements occur also in other parts of the cell, i.e., in the apical region as medium-sized vacuoles (Fig. 8) and under the plasmalemma and among rough-surfaced cisternae (Fig. 6) as clusters of small vesicles; but their contribution must be limited since they are few in number.

At the time this work was initiated, the studies of Rothschild (1963) and Dallner (1963) on the fractionation of rough and smooth microsomes from rat liver homogenates were the only comprehensive efforts to define the sedimentation characteristics of microsomal subfractions. When these techniques were applied to guinea pig pancreatic microsomes, however, no clear-cut separation was obtained.

Separation of rough from smooth microsomes was finally achieved by isopycnic centrifugation in a linear sucrose density gradient, the upper boundary of which had a density approximately equal to that of smooth microsomes (1.04 M sucrose; density, 1.14 g/cc, Rothschild, 1963), while the density at the bottom reached 1.27 g/cc (2.0 M sucrose), i.e., it was slightly higher than the estimated density of rough microsomes and zymogen granules. In such a gradient and at equilibrium, smooth microsomes were expected to remain on top or move only a small distance into it, whereas rough microsomes should band in the lower third of the gradient.

When 0.25 ml of a resuspension of the common microsomal pellet in 0.3 M sucrose was layered on such a gradient and centrifuged for 7½ hr at 123,000 *g*, three well defined bands formed. Band I, subsequently shown to consist mainly of smooth microsomes, occupied the upper 0.5 cm of the gradient; band II, containing free ribosomes and larger membrane-bounded elements, occupied 1 cm immediately beneath band I; band III, comprised of rough microsomes, occupied the next 1.5 cm of the gradient. The average density of band I was 1.145 g/cc and that of band III ranged from 1.180 to 1.230 g/cc. Increasing the time of centrifugation to 15 hr did not alter the position of bands I and III, but caused band II to move about 1 cm further down the gradient. Since the components of band II were not of interest in this study, no further attempts were made to resolve them.

Samples of bands I, II, and III were collected and pelleted as described in Methods. These pellets were examined to determine their structural constituents and chemical composition.

ELECTRON MICROSCOPY OF MICROSOMAL SUBFRACTIONS: *Band I.* The top third of the corresponding pellet consisted mainly of small, smooth-surfaced vesicles, each bounded by a unit membrane and each containing material of variable, usually low density (Fig. 9 *a*). The size range (45–75 mμ) of these vesicles was similar to that of the smooth-surfaced vesicles in the periphery of the Golgi region (cf. Fig. 7). The middle third of the pellet (Fig. 9 *b*) was comprised of a heterogeneous collection of smooth-surfaced vesicles and flattened cisternae. The larger vesicles were bounded by a unit membrane and appeared devoid of content; the smaller vesicles were similar to those of the upper third of band I in size. The majority of the flattened cisternae had an electron-opaque content. Rough-surfaced microsomes, recognizable by their attached ribosomes, were rare at this level in the pellet. The lower third of band I (Fig. 9 *c*) contained, in addition to smooth-surfaced vesicles and flattened cisternae, a few rough-surfaced

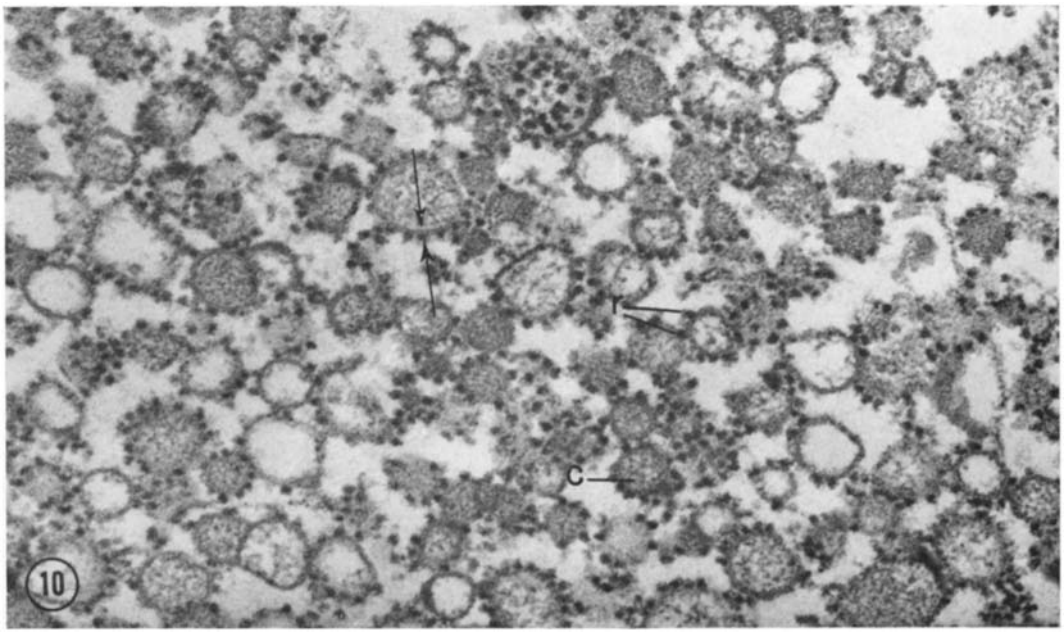

FIGURE 10 Micrograph through the mid portion of the rough microsomal pellet. Note that the majority of the microsomes is bounded by a continuous unit membrane (arrows) and is partly or completely filled with finely fibrillar, electron-opaque material (*c*). *r*, attached ribosomes. × 70,000.

contaminants which were stratified in the pellet at a depth corresponding to maximal centrifugal force.

The rough contaminants accounted for only a small percentage of the total content of band I; hence this band consisted primarily of smooth microsomes and is referred to as such hereafter.

Band II, the most heterogeneous of the three bands, consisted mainly of tightly packed free ribosomes, large, empty, rough-surfaced microsomes and some smooth microsomes.

Band III was the most homogeneous of the three bands: It consisted almost entirely of intact, rough-surfaced microsomes, practically all of which were bounded by an intact unit membrane and filled with a material of moderate electron opacity (Fig. 10). The rough microsomes in the lower half of the pellet were generally larger in diameter than those of the upper half. A few swollen, extracted mitochondria and smooth-surfaced vesicles contaminated the upper half of the pellet. This band is referred hereafter as rough microsomes.

CHEMICAL COMPOSITION OF MICROSOMAL SUBFRACTIONS: Table I gives the gross chemical composition of the total microsomal fraction and of microsomal subfractions isolated by isopycnic centrifugation. Corresponding data on rough and smooth microsomes isolated from liver homogenates (Dallner, 1963), and on total microsomes isolated from guinea pig (Palade and Siekevitz, 1956 *b*), dog (Hokin, 1955), and mouse (Van Lancker and Holtzer, 1959) pancreas are included for comparison.

The total microsomal fraction isolated from incubated slices has an RNA and protein content similar to that of microsomes prepared from fresh guinea pig pancreas (Palade and Siekevitz, 1956 *b*) and from the pancreas of the dog (Hokin, 1955) and mouse (Van Lancker and Holtzer, 1959). The phospholipid-phosphorus/protein ratio of slice microsomes is also similar to that reported for guinea pig pancreatic microsomes but is considerably lower than that found in dog pancreatic microsomes.

The RNA/protein ratio of smooth microsomes (band I) ranged from 0.05 to 0.09. As indicated by electron microscopy, this low ratio reflects the minimal contamination of the fraction by rough microsomes. The RNA/protein ratio of rough microsomes (band III) is somewhat greater than that of the starting material as would be expected

TABLE I

Chemical Composition of Microsomal Fractions and Subfractions

Source	Total microsomes	Smooth microsomes (Band I)	Rough microsomes (Band III)	Band II
		mg RNA/mg protein		
Guinea pig pancreatic slices*	0.28–0.31 (5)	0.05–0.09 (5)	0.39–0.44 (5)	0.43 (avg, 5)
Guinea pig pancreas‡	0.10–0.22			
Dog pancreas§	0.18			
Mouse pancreas‖	0.20–0.24			
Rat liver¶	0.21	0.05	0.33	
		μg phospholipid phosphorus / mg protein		
Guinea pig pancreatic slices*	4.3 (avg, 5)	6.0–6.7 (2)	3.6–5.2 (2)	4.5 (avg, 2)
Guinea pig pancreas‡	2.25–4.8			
Dog pancreas§	10.5			
Rat liver¶	9.6	14.7	6.4	

* Number of experiments indicated in brackets.
‡ Palade and Siekevitz, 1956 *b*.
§ Hokin, 1955.
‖ Van Lancker and Holtzer, 1959.
¶ Dallner, 1963.

upon removal of smooth microsomes. Band II is similar to band III in RNA content.

The concentration of phospholipid in smooth microsomes is about two times that in rough microsomes. The phospholipid concentration of band II is similar to that of band III. In contrast to the smooth, rough, and total microsomal fractions isolated from liver, the corresponding pancreatic fractions have a lower phospholipid concentration (relative to protein) reflecting the lower content of phospholipid in pancreatic homogenates (Palade and Siekevitz, 1956 *a*, *b*).

The total pelleted material derived from bands I, II, and III from one gradient generally amounted to ~1.0 mg protein, band I accounting for ~20%, band II for ~35%, and band III for ~45% of the total. The protein recovered in the pelleted bands comprised ~50% of the initial load of microsomes on the gradient. Total recovery of the bands was sacrificed in favor of collecting representative samples of each band uncontaminated by components of adjacent bands.

KINETICS OF LABELING OF MICROSOMAL SUBFRACTIONS: To determine the kinetics of labeling of microsomal subfractions and thereby check the postulated transfer of secretory proteins from the rough ER to small vesicles at the periphery of the Golgi complex, we performed experiments in which rough and smooth microsomes were isolated as described from sets of slices at the end of a 3-min pulse labeling with L-leucine-^{14}C (1 μc/ml; 4.0 μM) and after further 7-, 17-, and 57-min incubation in chase medium (L-leucine-^{12}C, 3 mM). Since the SW39 rotor holds only three gradient tubes at a time, the experiments were performed in the groupings shown in Table II. Each experiment was performed on the sliced pancreas of a single animal. In all experiments but two (3-min pulse and 17-min chase), pulse labeling and chase incubation were performed in medium with complete amino acid supplementation.

The results are presented in Table II. The specific radioactivity of TCA-precipitable proteins in the smooth, rough, and total microsomal fractions are tabulated at the end of the pulse (3-min) and after various times of incubation in chase medium.[3] In addition, the specific radioactivity of the soluble proteins in the post-microsomal supernate is given. Since the specific radioactivity of microsomes at the end of the pulse varied somewhat between experiments, the results are also expressed as specific radioactivity ratios of smooth to rough microsomes to facilitate comparison.

[3] The duration of post-pulse incubation in chase medium is designated +7 min, +17 min, etc.

TABLE II

Specific Radioactivities of Proteins in Microsomal and Submicrosomal Fractions and in the Post-Microsomal Supernate

Exp. no.	Incubation time		Total microsomes cpm/mg protein	Smooth microsomes cpm/mg protein	Rough microsomes cpm/mg protein	SA smooth / SA rough	Post-microsomal supernate cpm/mg protein
	Pulse	Chase					
	min	*min*					
1	3		2750	2220	5080	0.44	—
	3	+ 7	2090	5100	2480	2.06	—
	3	+17	1450	2580	1850	1.39	—
2	3		2180	1680	2740	0.61	360
	3	+ 7	2020	3500	1670	2.10	470
	3	+17	1365	1850	1350	1.37	440
3	3		3380	1720	3950	0.43	390
	3	+17	3510	3770	2050	1.85	650
4	3		3670	2390	5370	0.44	213
	3	+17	3200	5780	2590	2.23	450
5	3		3050	980	4540	0.22	300
	3	+17	1950	3480	2030	1.71	480
6	3		1860	470	2310	0.20	210
	3	+17	2140	1612	2420	0.65	280
7	3		1370	920	2300	0.40	370
	3	+17	1070	2000	1120	1.78	460
8	3		3400	1460	3800	0.38	990
	3	+17	2530	3770	2300	1.64	970
	3	+57	1750	2190	1470	1.49	930

In every experiment, the specific radioactivity of the smooth microsomes was less than that of the rough (and total microsomes) at the end of the pulse labeling. This is indicated by an average specific radioactivity ratio of smooth to rough of 0.39.

After 7 min of incubation, the specific radioactivity of smooth microsomes was greater than that of rough microsomes (specific radioactivity ratio of smooth to rough = 2.08). Although this ratio was due in part to a decrease of the specific radioactivity of rough microsomes, it also reflects an absolute increase of the specific radioactivity of smooth microsomes. After +17 min of incubation, the situation was similar to that found after +7 min except that both the absolute specific radioactivity of the smooth fraction and the specific radioactivity ratios of smooth to rough microsomes (1.56 average) were somewhat less than after +7 min. In one experiment (No. 6), the specific radioactivity ratio at +17 min was only 0.65, and the specific radioactivity of the rough microsomes also increased at +17 min; these discordant results were probably due to an inefficient chase. In most experiments, the specific radioactivity of the total microsomal fraction decreased during chase incubation paralleling the decrease in specific radioactivity of the rough microsomes.

In experiment 8, smooth and rough microsomes were isolated after both +17 and +57 min of incubation. The specific radioactivity of the smooth microsomes at +17 min was greater than that at either 3 min or +57 min. In contrast, the specific radioactivity of both rough and total microsomes progressively decreased during chase incubation. Since at later times (> +17 min) the specific radioactivity of smooth microsomes decreased in parallel with that of the rough

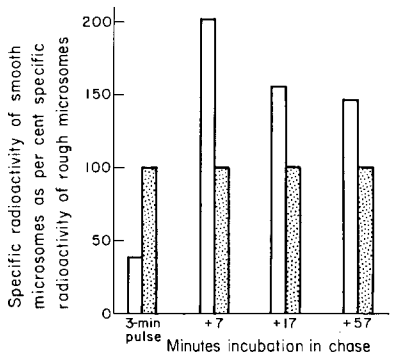

FIGURE 11 Graphical presentation of data from Table II. Open bars: smooth microsomes. Stippled bars: rough microsomes.

microsomes, no further information was sought by the performance of subfractionation experiments at either intermediate times (between +17 and +57 min) or times greater than +57 min.

Data from the four time points examined are also shown graphically in Fig. 11. To permit averaging, we have calculated the specific radioactivities of the smooth microsomes as percentages of the specific radioactivities of the corresponding rough microsomes.

In general, the specific radioactivity of proteins in the post-microsomal supernatant was about 10x less than that of particulate fractions (except for experiment 8) and remained constant or rose slightly during incubation in chase medium.

For reasons given above, band II was not routinely collected and assayed. In all experiments, changes in its specific radioactivity reflected closely those of band III, as was expected since rough microsomes are a major component of this subfraction.

If changes in total radioactivity in the proteins of smooth and rough microsomes are considered, a pattern similar to the changes in specific radioactivities emerges (data not shown). The absolute loss of radioactive proteins from rough microsomes between the end of the pulse and +7 min of incubation in chase medium can be accounted for largely by gains in radioactive protein in the smooth microsomal fraction. By +17 min, the net gain of radioactivity in the smooth microsomes accounted for only about 75% of that lost by the rough microsomes, suggesting that some of the radioactivity had already reached the zymogen granule fraction (see the following paper).

Location of Labeled Protein in Microsomal Subfractions

1. MILD ALKALINE EXTRACTION OF MICROSOMAL SUBFRACTIONS: In view of the basic assumption that we are dealing with secretory proteins in transit from one cell compartment to another, it was important to determine whether the radioactive proteins associated with both the rough and smooth microsomes were contained in the cavities of the microsomal vesicles or incorporated in their membrane. A similar problem has been dealt with by Greene et al. (1963) in relation to a fraction of zymogen granules isolated from bovine pancreas. They demonstrated that 85% of the proteins of the fraction, mostly digestive enzymes, could be extracted by incubation in isotonic NaCl-$NaHCO_3$ at pH 8.4; found that the sedimentable material which remained after extraction consisted mainly of empty granule membranes; and concluded from this evidence that the digestive enzymes were located in the granule content. Lysis of dog zymogen granules at pH greater than 7.0 had been previously reported by Hokin (1955). When the same extraction procedure was applied to rough and smooth microsomes isolated from incubated pancreatic slices (Table III), the results showed that treatment with NaCl-$NaHCO_3$, pH 8.4, releases from all the microsomal fractions, and especially from the smooth fraction, the majority of the TCA-precipitable radioactivity (71–85%) while removing only about 18–28% of the original protein of the pellets. Accordingly, the specific radioactivity of the released protein is considerably higher than that of either the original pellet or the extraction residue which consists primarily of empty vesicles (microsomal ghosts). In the case of the zymogen granule fraction, a major portion of both the radioactivity (83%) and contained protein (64%) is released and this results in a relatively smaller increase in the specific radioactivity of the extracted protein.

These results indicate that the majority of the radioactive protein in both microsomal subfractions and the zymogen granule fraction is contained within the cavity of the particles rather than being attached to, or part of, their limiting membranes. Further, since the specific activity of the released protein is greater than that of the remaining sedimentable fraction (which, in a sense, represents the nonexportable proteins of the

TABLE III

Release of Protein and Radioactivity from Microsomal Subfractions and Zymogen Granule Fraction by Alkaline Extraction

	Time in chase	Untreated pellet cpm/mg protein	Protein released by treatment	Radioactivity released by treatment	Released protein (extract) cpm/mg protein	Extraction residue cpm/mg protein
	min		%	%		
Smooth microsomes	+17	34,000	28.3	84.6	114,000	8,250
Rough microsomes	+17	19,400	18.2	71.0	37,000	7,800
Zymogen granule fraction	+57	34,300	64.2	83.0	43,600	16,000

Microsomal subfractions were isolated from slices pulse labeled with 2.0 μc/ml L-leucine-^{14}C (1.6 μM) in Krebs-Ringer bicarbonate medium containing a full amino acid supplement, and incubated +17 min in chase containing L-leucine-^{12}C, 3.5 mM. Zymogen granules were isolated from slices (separate experiment) pulse labeled with L-leucine-^{14}C, 1.0 μc/ml; 0.8 μM (same medium as above), and incubated +57 min in chase containing L-leucine-^{12}C, 3.5 mM. Duplicate pellets of each fraction were collected. One set served as the untreated control; the other was resuspended in 1.0 ml 0.17 M NaCl to which was added 5.0 ml 0.2 M $NaHCO_3$, pH 8.4. After incubation at 0° for 45 min, the extract and extraction residue were separated by centrifuging for 2 hr at 105,000 *g*.

cell), the released proteins have the expected characteristics of secretory proteins: as shown by Siekevitz and Palade (1960), the rate of labeling of secretory proteins is ~ five to seven times greater than that of proteins retained in the cell.

2. ELECTRON MICROSCOPIC RADIOAUTOGRAPHY OF THE SMOOTH MICROSOMAL FRACTION: As already indicated, the smooth microsomal subfraction is contaminated by a small amount of rough microsomes. To determine the contribution of the latter to the total radioactivity in the subfraction, we performed electron microscopic radioautography on thin sections of smooth microsomes isolated from a set of slices which had been pulse labeled for 3 min with L-leucine-^{3}H (200 μc/ml; 40 μM) and incubated in chase medium (L-leucine-^{1}H, 2 mM) for a further +17 min. Sections were cut through the depth of the pellet, mounted on slit grids, and coated with photographic emulsion as described by Caro and van Tubergen (1962). After exposure and photographic processing, serial electron micrographs were taken down the entire depth of the pellet. Each silver grain in the micrographs was scored as to its association with rough or smooth microsomes. Grain counts indicated that the radioautographic label was almost exclusively (~90%) localized to smooth microsomes so that, at most, only ~10% of the radioactivity in the smooth microsomal fraction at +17 min can be accounted for by contaminating rough microsomes.

DISCUSSION

Previous cell fractionation studies carried out on the pancreas after in vivo labeling have shown that radioactive amino acids are initially incorporated into the secretory proteins of the microsomal fraction (Siekevitz and Palade, 1960), more precisely into the secretory proteins of attached ribosomes (Siekevitz and Palade, 1960; Redman et al., 1966); subsequently, labeled secretory proteins appear in other cell fractions—notably zymogen granules. These studies suggested that secretory proteins are synthesized in the rough ER and transferred, perhaps through the cisternal spaces, to zymogen granules, but other interpretations were not excluded. Caro and Palade (1964) demonstrated by electron microscopic radioautography, again after in vivo labeling, that secretory proteins synthesized in the rough ER pass through the periphery of the Golgi region in transit to the condensing vacuoles of the Golgi complex where they are intensively concentrated, the vacuoles becoming, in the process, zymogen granules. The periphery of the Golgi region is characterized by the presence of transitional rough ER elements (part rough and part smooth) and of clusters of smooth-surfaced vesicles. Because of limited radioautographic resolution, proof that these vesicles function in the transport of secretory proteins from rough ER to condensing vacuoles could not be obtained. Evi-

dence implicating the general area of the Golgi complex in intracellular transport and zymogen granule formation was also obtained by Warshawsky et al. (1963) and by van Heyningen (1964), but alternative views of this part of the secretory process have been proposed. Laird and Barton (1958), Morris and Dickman (1960), and Redman and Hokin (1959) have suggested that secretory proteins leave the microsomes and pass, in soluble form, through the cytoplasmic matrix into zymogen granules or directly into the glandular lumen, while Sjöstrand (1962) postulated (on the basis of morphological findings only) that elements of the Golgi complex are concerned with both the synthesis and segregation of secretory proteins.

Presently available data obtained by cell fractionation or electron microscopic radioautography indicate, therefore, that synthesis of secretory proteins takes place in the rough ER, that the secretory proteins move through the Golgi complex where their presence is reliably ascertained in condensing vacuoles, and that they are finally stored in zymogen granules.

Clearer insight into the sequence of events involved in synthesis and intracellular transport could not be obtained in these studies because true pulse labeling of secretory proteins with radioactive amino acids was not possible in the whole animal, a situation which affected the interpretation of kinetic data obtained by fractionation as well as radioautography. Furthermore, cell fractionation was imperfect—there was extensive intercontamination of fractions; and incomplete—a number of subcellular components were unaccounted for. This applied especially to the Golgi complex which appears to be extensively involved in a number of operations in the secretory cycle. Finally, limitations of radioautographic resolution prevented definite conclusions when structures smaller than $\sim 0.2\ \mu$ were involved.

To overcome the limitations inherent to the investigation of the pancreas in vivo, we developed a system of guinea pig pancreatic slices incubated in vitro. The main characteristics and advantages of this system are as follows.

1. Pancreatic slices actively function for as long as 3 hr in vitro as indicated by the kinetics of amino acid incorporation into protein. This is well within the time required to complete all phases of synthesis and intracellular transport of secretory proteins in vivo. During incubation there is minimal cell damage as determined by electron microscopy, and thus cell fractions isolated from the slices are cytologically as valid as those derived from the gland in vivo.

2. In contrast to the whole animal in which pulses of less than 15 min cannot be achieved, short, well defined pulse labeling of proteins can be obtained in slices. Post-pulse incubation in a chase medium, in which the level of unlabeled amino acid can be controlled, allows a dilution of the tracer up to 1000x, and results in rapid cessation of further incorporation of label. During chase incubation the label is conserved in protein. Because of short, stable pulse labeling, post-pulse changes in the location of label in the cell can be due only to intracellular transport rather than continued synthesis. The latter possibility could not be ruled out in all previous studies carried out on the whole animal.

3. As a practical consideration, the slice system allows us to carry out a complete kinetic experiment at three or four time points on the sliced pancreas of a single animal, thus avoiding individual variations in activity.

For the present study, it was also necessary to overcome the two limitations of existing cell fractionation techniques pointed out above: the imperfect and incomplete character of the fractionation.

The first problem, imperfect fractionation, i.e., extensive intercontamination of the fractions, is the result of the intrinsic limitations of differential centrifugation. To contend with this situation, we abandoned any attempt at a systematic recovery of cell fractions and, instead, concentrated on obtaining representative fractions of reasonably purity. For instance, prior to isolation of the total microsomal fraction, larger cell components were sequentially removed by differential centrifugation, with the result that considerable losses of microsomes occurred. The total microsomal fraction was further purified by the use of equilibrium centrifugation in a density gradient, resulting in the isolation of acceptably homogeneous fractions of rough- and smooth-surfaced microsomes. Using the same rationale, we obtained an acceptably homogeneous zymogen granule fraction at the expense of considerable losses into the preceding and following fractions (see next paper).

To cope with incomplete fractionation, i.e.,

absence of a distinct fraction for each recognizable subcellular component, we concentrated only on those structural components directly involved in the synthesis and intracellular transport of secretory proteins and disregarded those not directly involved in these processes. Using this approach, we have successfully isolated a fraction representative of the protein-synthesizing elements of the cell (rough microsomes derived from the rough ER), and have obtained a fraction (smooth microsomes) which contains the small vesicles and fragmented cisternae of the Golgi complex. As will be seen in the following paper, we have determined by electron microscopy that the condensing vacuoles of the Golgi complex sediment in the zymogen granule fraction.

Pulse-labeling experiments, in which the kinetics of transport of labeled proteins from the rough- to the smooth-microsomal fraction have been followed, provide direct evidence that newly synthesized secretory proteins are transferred, in time, from the cisternae of the rough ER to the small vesicles of the Golgi complex. Thus, these small vesicles represent a station in the intracellular transport of secretory proteins. Although these findings apply directly to smooth microsomes, morphological evidence indicates that the main source of such particles in the exocrine cell is the Golgi complex.[4] Previous radioautographic observations (Caro and Palade, 1964) had already implicated the periphery of the Golgi region in transfer; our experiments indicate that definite structures present in this region, i.e., the small, smooth-surfaced vesicles, are involved in this operation. Finally, the results of extraction experiments performed in microsomal subfractions indicate that the newly synthesized secretory proteins are located primarily within the content of both rough and smooth microsomes rather than being associated with the bounding membrane. From this, we can conclude that following segregation in the cisternal spaces of the rough ER, secretory proteins remain within and are transported through membrane-enclosed spaces of the exocrine cell. In the following paper, the results of radioautographic and cell fractionation experiments performed on slices during longer times of incubation in the chase medium will demonstrate the remaining steps in the intracellular transport of pulse-labeled secretory proteins, viz., transport from the peripheral elements of the Golgi complex into condensing vacuoles, zymogen granules, and, finally, the duct system of the gland.

The specific radioactivity of proteins in the post-microsomal supernate, which reflects mainly the specific activity of soluble proteins in the cytoplasmic matrix (cell sap) remains reasonably constant throughout incubation (i.e., up to +57 min of incubation), indicating negligible equilibration between the labeled proteins of particulate fractions and those of the cytoplasmic matrix. The significance of this result will be discussed in the following paper.

[4] Exocrine cells, in turn, account for 85% of the volume of the mammalian pancreas (Ogilvie, 1933). Consequently, cell fractions isolated from homogenates of the total gland must be derived largely from exocrine cells.

Part of this work was supported by a United States Public Health Service Research Grant AM 10928-01.

Received for publication 20 February 1967.

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