

COMPARATIVE STUDIES OF INTRACELLULAR TRANSPORT OF SECRETORY PROTEINS

A. TARTAKOFF and P. VASSALLI, with the technical assistance of M. DÉTRAZ

From the Department of Pathology, University of Geneva, Faculty of Medicine, 1211 Geneva 4, Switzerland

ABSTRACT

The physiology of protein intracellular transport and secretion by cell types thought to be free from short-term control has been compared with that of the pancreatic acinar cell, using pulse-chase protocols to follow biosynthetically-labeled secretory products. Data previously obtained (Tartakoff, A. M., and P. Vassalli, *J. Exp. Med.* **146**:1332-1345) has shown that plasma-cell immunoglobulin (Ig) secretion is inhibited by respiratory inhibitors, by partial Na/K equilibration effected by the carboxylic ionophore monensin, and by calcium withdrawal effected by the carboxylic ionophore A 23187 in the presence of ethylene glycol bis (β -aminoethylether)-*N,N,N',N'*-tetraacetic acid (EGTA) and absence of calcium. We report here that both inhibition of respiration and treatment with monensin slow secretion by fibroblasts, and also macrophages and slow intracellular transport (though not discharge per se) by the exocrine pancreatic cells. Attempted calcium withdrawal is inhibitory for fibroblasts but not for macrophages. The elimination of extracellular calcium or addition of 50 mM KCl has no major effect on secretory rate of either fibroblasts or macrophages.

Electron microscopic examination of all cell types shows that monensin causes a rapid and impressive dilation of Golgi elements. Combined cell fractionation and autoradiographic studies of the pancreas show that the effect of monensin is exerted at the point of the exit of secretory protein from the Golgi apparatus. Other steps in intracellular transport proceed at normal rates.

These observations suggest a common effect of the cytoplasmic Na/K balance at the Golgi level and lead to a model of intracellular transport in which secretory product obligatorily passes through Golgi elements (cisternae?) that are sensitive to monensin. Thus, intracellular transport follows a similar course in both regulated and nonregulated secretory cells up to the level of distal Golgi elements.

KEY WORDS exocrine pancreas · fibroblasts · Golgi apparatus · intracellular transport · ionophore · macrophage · secretion

The physiology of protein secretion by cells whose rate of secretion is thought to be free from short-term control has been rarely studied. By contrast

with cells which secrete only when appropriately stimulated, such "nonregulated" secretory cells lack a conspicuous storage compartment for secretory protein. Examples of such cells are plasma cells, fibroblasts, and macrophages.

In a previous publication, we have explored the secretory physiology of normal and malignant

plasma cells (31). We found no evidence for the existence of short-term physiological control of the rate of discharge of Ig nor other behavior characteristic of regulated secretory cells (e.g., dependence on extracellular calcium or responsiveness to either increased intracellular calcium or extracellular KCl). All of the data indicate that the ongoing "basal" secretory rate is maximal. Three conditions were identified which lower secretory rate: block of respiration, partial equilibration of cellular Na/K levels effected by the carboxylic ionophore monensin, and cellular calcium depletion as effected by the carboxylic ionophore A 23187 in the presence of ethylene glycol bis(β -aminoethyl ether)*N,N,N',N'*-tetraacetic acid (EGTA) and the absence of calcium. By electron-microscopic-autoradiographic studies, an energy-dependent step in the secretory cycle has been identified at the rough endoplasmic reticulum (RER)-Golgi junction. Moreover, both ionophores appear to perturb the ongoing traffic of Golgi-derived vesicles. In the case of monensin, a storage compartment for secretory protein becomes apparent—an impressively abundant population of smooth-surfaced vacuoles whose dimensions resemble those of storage granules in "regulated" secretory cells. In the case of calcium depletion, the Golgi lesion results in an extensive accumulation of small vesicles.

In the present study, we explore the generality of these observations and compare the secretory physiology of the plasma cell with that of fibroblasts (which secrete procollagen) and activated macrophages (which secrete a mixture of proteins and enzymes). We also investigate the extent to which the physiology of discharge by such seemingly nonregulated secretory cells resembles the physiology of intracellular transport in a well-characterized "regulated" secretory cell—the acinar cell of the exocrine pancreas.

MATERIALS AND METHODS

The materials used and the labeling and analytic procedures employed are modeled after those of our previous studies (31).

Activated macrophages were obtained from peritoneal washings of C3H/C57 mice injected intraperitoneally 3 days previously with 2 ml of 3% thioglycollate broth. They were plated on Falcon Petri dishes (Falcon Plastics, Division of BioQuest, Oxnard, Calif.) at 2×10^6 cells/cm² in 5% fetal calf serum in Dulbecco-modified Eagle's medium (5). After overnight culture, non-adherent cells were removed by repeated washings with Hanks' basic salt solution. Cells were labeled with

[³H]leucine (or [³⁵S]methionine) at 100 μ Ci/ml in medium containing 8 nM leucine (or simply lacking non-radioactive methionine), 20 μ g/ml bovine serum albumin, nonessential amino acids, and 10 mM N-2-hydroxyethyl-piperazine-N'-2-ethane sulfonic acid (Hepes). After labeling, the cells were rinsed with Hanks' basic salt solution and chased in appropriate media (variants of Dulbecco Modified Eagle's Medium). After suitable chase intervals, the media were recovered, cells were lysed in Hanks' solution which contained 0.1% Nonidet P-40, and both samples were centrifuged at 1,000 g for 5 min before removing aliquots for counting.

Chick embryo tendon fibroblasts were obtained from 17-day-old chick embryos, as in the procedure of Dehm and Prockup (6). Briefly, tendons were digested with a mixture of crude trypsin and collagenase for 90 min at 37°C. The liberated cells were washed in the presence of fetal calf serum and soy bean trypsin inhibitor, labeled 1 h at 37°C with 100 μ Ci/ml [³H]proline, washed, and returned to appropriate chase media. After the 90 min secretion interval, the cell supernates and detergent lysates of the cells (see macrophage procedure) were recovered, adjusted to 1 mM N-ethylmaleimide, 1 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride to block proteolysis, and centrifuged, as in the macrophage procedure. Secretion from fibroblasts or macrophages was either precipitated with 5% TCA on filter disks (for counting), or precipitated with carrier formalinized Staphylococci plus TCA as preparation for sodium dodecyl sulfate (SDS)-gel electrophoresis. The use of carrier bacteria was as effective as 1 mg/ml carrier albumin and avoided overloading the gels at the 68,000-dalton level. (The bacteria remain largely insoluble in sample buffer).

Guinea pig pancreatic lobules were prepared by standard procedures (27). Cell fractionation was according to Tartakoff and Jamieson (30), except that the post-mitochondrial supernate was adjusted to 1.3 M sucrose and loaded into a "sandwich gradient" (0.5 ml 2.2 M sucrose, 0.5 ml 1.3 M sucrose, 1.5 ml post-mitochondrial supernate, 0.5 ml 1.2 M sucrose, 0.5 ml 0.3 M sucrose) which was spun 18 h at 250,000 g in the Spinco SW56 rotor (Beckman Instrument, Inc., Spinco Div., Palo Alto, Calif.). The rough and smooth microsomal fractions were collected and washed once with 0.3 M sucrose, 100 mM KCl (1 h at 150,000 g in the Spinco Ti 50 rotor) to eliminate the considerable background of adsorbed secretory protein (28). Protein was determined by the method of Lowry et al. (18) with bovine albumin as a standard.

Gradient SDS gels were run by the procedure of Laemmli (16). Samples were routinely dissolved and reduced in the presence of 1% SDS, 10 mM β -mercaptoethanol, by heating 2 min at 100°C before loading. Fluorography of dried gels was performed according to Bonner and Laskey (4).

Tissue fixation and autoradiography were performed as in (31).

Guinea pigs and 17-day-old chick eggs were obtained

from local sources. All mice were (C3H × C57)_F₁ hybrids. Fluid thioglycollate broth and trypsin were obtained from Difco Laboratories, Detroit, Mich. Soybean trypsin inhibitor, collagenase (*Cl. Histolyticum*), phenylmethyl sulfonyl fluoride, and N-ethyl maleimide from Sigma Chemical Co., St. Louis, Mo.; fetal calf serum from Gibco Diagnostics, The Mogul Corp., Chagrin Falls, Ohio; L-[³H(G)]proline 5 Ci/mmol, and L-[4,5-³H(N)]leucine 50 Ci/mmol from New England Nuclear, Boston, Mass.; L-[³⁵S]methionine 800 Ci/mmol from Amersham Corp., Arlington Heights, Ill.; monensin and A23187 from R. L. Hamill of Eli Lilly and Co., Indianapolis, Ind.; and carboxyl cyanide m-chlorophenyl hydrazone from Calbiochem, San Diego, Calif. All other chemicals were of reagent grade.

RESULTS

Fibroblasts

Cells were routinely labeled for 1 h, washed, and returned to nonradioactive medium for 90 min chase. Analysis on SDS gels of either the total intracellular material or secretion (both after reduction) revealed the presence of two predominant bands of radioactivity, corresponding to the procollagens α_1 and α_2 in mobility; as expected these were present in a proportion roughly judged to be 2:1 (Fig. 1).

Fig. 2 illustrates the influence of energy block and ionic perturbations on release of TCA-insoluble radioactivity during the 90-min chase interval.

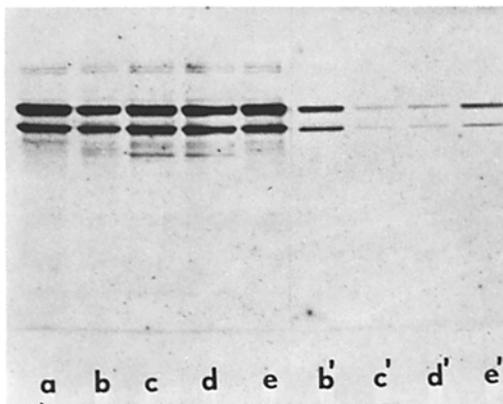


FIGURE 1 5–20% SDS gel of samples of reduced fibroblast secretion and cytoplasmic extracts. In all samples the fluorogram reveals two principal bands; a–e: intracellular, b'–e': extracellular. (a) no chase; (b) control chase; (c) CCCP, 10 μ M; (d) monensin, 1 μ M; (e) A23187, 10 μ M; EGTA, 1 mM in calcium-free medium. In the extracellular samples note that CCCP or monensin, and to a lesser extent A23187-Ca, are inhibitory.

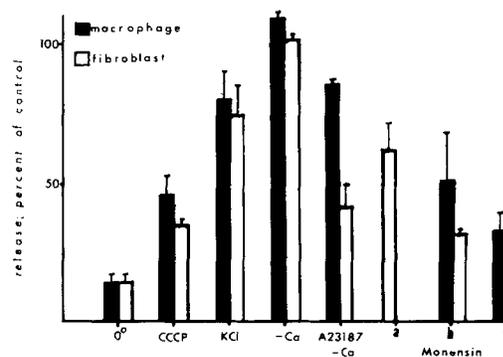


FIGURE 2 Quantitation of secretion by fibroblasts and macrophages under various conditions. Details are given in Methods. Fibroblasts were labeled for 1 h with [³H]proline and allowed to secrete for 90 min. Macrophages were labeled for 1 h with [³H]leucine and allowed to secrete for 1 h. All data is normalized to controls maintained at 37°C. For control fibroblasts, during the 90 min secretion interval 54% of the initial cellular TCA-insoluble radioactivity was released. Indications on the abscissa: CCCP, 10 μ M CCCP in glucose-free medium; KCl, 50 mM KCl replaces an equivalent amount of NaCl; -Ca, calcium-free medium; A23187-Ca, 10 μ M A23187, 1 mM EGTA in calcium-free medium; monensin (a) 1 μ M, (b) 10 μ M, (c) 10 μ M monensin present during both labeling and chase intervals. Standard deviations are indicated.

Extracellular calcium was not needed to maintain maximal secretory rate, and excess KCl was not stimulatory. A partial SDS-gel analysis of such an experiment is included in Fig. 1. The inhibition due to monensin carboxyl cyanide m-chlorophenyl hydrazone (CCCP), or A23187 in the presence of EGTA and absence of calcium is evident. A23187 in the presence of calcium was toxic.

Macrophages

Activated macrophages were routinely labeled for 1 h with [³H]leucine, then thoroughly washed and returned to nonradioactive "chase" medium. The kinetics of the release of TCA-insoluble counts per minute is given in Fig. 3. It is evident that secretion is rapid; if maximal release is taken to correspond to 30% of the incorporated radioactivity (the level attained after 20 h chase), one-half of this level is attained after ~1 h chase.

The mixture of proteins released has been examined by SDS-gel electrophoresis after reduction (Fig. 4). The fluorographic image is complex and spans a broad mol-wt range, from 14,000 daltons (lysozyme [10]) to proteins that scarcely enter the running gel, among which are presumably the

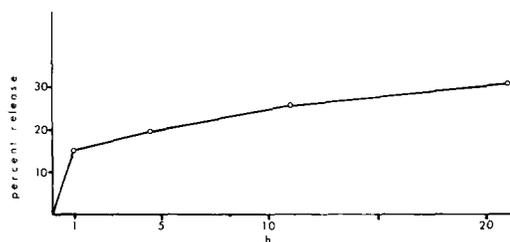


FIGURE 3 Kinetics of secretion of TCA-insoluble radioactivity from macrophages prelabeled for 1 h with [^3H]leucine. 100% equals total initial intracellular TCA-insoluble radioactivity before chase.

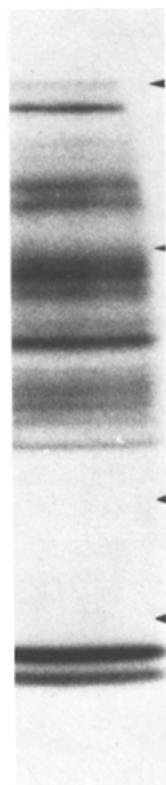


FIGURE 4 10–15% SDS gel of macrophage secretory proteins labeled with [^{35}S]methionine. Cells were labeled for 1 h, washed, and allowed to secrete for 1 h. The extracellular medium was recovered, precipitated with TCA, reduced, and loaded onto the gel. Arrows designate, from top to bottom, the top of the running gel, the mobility of BSA, trypsinogen, and ribonuclease. Similar gels of intracellular samples excluded cell lysis as an important contributor to secretion.

collagenase, elastase, and plasminogen activator recently described (32–34). No attempt has been made to determine whether these multiple pro-

teins are released in synchrony.

Because half-maximal discharge occurs within 1 h, this chase interval has been selected for evaluation of the energy requirement and of the influence of various ionic perturbations on discharge rate. Protein synthesis continues in the presence of monensin (not shown), and therefore monensin was present during both the 1-h labeling period and the 1-h chase interval in certain experiments. Fig. 2 shows that inhibition by monensin was modest when the ionophore was present only during the chase period, but more pronounced when it was also present during the labeling. Energy starvation was also strongly inhibitory, though this effect was only slight if glucose was present, thus suggesting an important glycolytic contribution to ongoing metabolism. In these cases of inhibition, gel analysis has shown the entire spectrum of secretory proteins to be effected (data not shown).

Neither elimination of extracellular calcium nor an attempt at depletion of cytoplasmic calcium was substantially inhibitory, (nevertheless, treatment with A23187 and EGTA profoundly inhibits protein synthesis—data not shown). An excess of 50 mM KCl in the medium was slightly inhibitory.

Exocrine Pancreas

It is known that ongoing metabolism is required both for discharge of secretory proteins by the exocrine pancreas and for intracellular transport from the RER to the Golgi complex (12, 14). Furthermore, discharge can be triggered by A23187 (when calcium is present) (9) or by excess KCl (25). It is not known whether partial Na/K equilibration or cytoplasmic calcium levels influence intracellular transport.

In a first set of experiments, we have explored the influence of ionophores on discharge. Pancreatic lobules were pulse-labeled for 10 min with [^3H]leucine, returned to chase medium for 3 h to concentrate radioactivity in the zymogen granules, and then incubated for 3 h under a variety of potentially stimulatory conditions. Fig. 5a shows the anticipated massive stimulation of discharge by carbamylcholine and the lack of influence of μM monensin either on basal or stimulated secretion. A gel analysis of secretion collected in the presence of carbamylcholine with and without monensin shows the two to be identical (data not shown). Although it is not included in the figure, the effect of 10 μM A23187 simulated that of carbamylcholine so long as calcium was present in

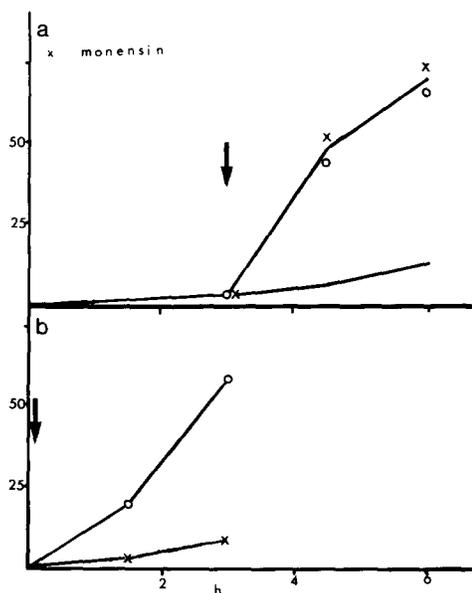


FIGURE 5 Evaluation of the influence of $1 \mu\text{M}$ monensin on the efficiency of exocrine pancreatic secretion. In panel (a) lobules were pulse labeled 10 min, washed, and returned to nonradioactive medium for 3 h to concentrate radioactivity in the zymogen granules. At this time (arrow), 10^{-5} M carbamylcholine was added, with (x) or without (o) monensin ($1 \mu\text{M}$). The illustration shows that over the ensuing 3-h period the secretory rate was not influenced by the presence of the ionophore. Also given is the control secretory rate at 37°C in the absence of stimulant. A comparably low level of release was observed when monensin was present in the absence of carbamylcholine. In panel (b) carbamylcholine was added immediately post-pulse (arrow). It is evident that under these conditions, monensin greatly slows the secretory rate.

the bathing medium and was not chelated by EGTA.

When comparable experiments were performed in which the 3-h post-pulse interval was omitted, the influence of monensin was strikingly different (Fig. 5b). Evidently, monensin blocks the release of newly synthesized protein, but not of protein already concentrated in zymogen granules.

Cell fractionation experiments have therefore been performed to identify the site(s) of intracellular transport sensitive to monensin. Fig. 6c shows the influence of monensin on transport of pulse-labeled radioactive proteins from the RER to the zymogen granule fraction (composed primarily of condensing vacuoles and zymogen granules [13]). $1 \mu\text{M}$ doses of monensin effectively slow the arrival of newly synthesized protein,

thereby adequately explaining the failure of such protein to be discharged by carbamylcholine stimulation. Accordingly, monensin action might be slowed at two possible sites; either at the point of exit of secretory protein from the RER, or at the passage from the Golgi complex to condensing vacuoles. Some interdependence or coupling between these two events might also be anticipated.

To distinguish among these possibilities, pancreatic lobules were pulse labeled 5 min and either held at 0°C or reincubated (in the presence of cycloheximide) for 1 h with or without $1 \mu\text{M}$ monensin. Homogenates were then prepared, and rough and smooth microsomes were isolated, taking care to eliminate adsorbed secretory proteins. Fig. 6a, b illustrates the relative specific activities of the fractions as a function of time. It is evident that drainage from the rough microsomes occurs at control rates in the presence of monensin. By contrast, there is an accumulation of TCA-insoluble radioactivity in the smooth microsomal sub-fraction instead of the decrease which normally follows efficient drainage to more distal compartments (12).

In similar cell-fractionation experiments, 10

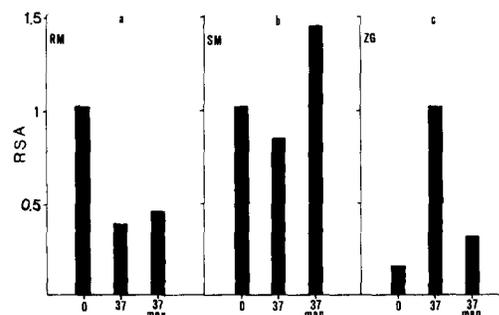


FIGURE 6 Influence of monensin on intracellular transport. Pancreatic lobules were pulse labeled for 5 min with $[^3\text{H}]$ leucine, washed, and transferred to media containing $5 \mu\text{g}/\text{ml}$ cycloheximide at 0°C , at 37°C , or at 37°C in the presence of $1 \mu\text{M}$ monensin. After 90 min, each set of lobules was homogenized and rough and smooth microsomal and zymogen granule fractions isolated. The TCA-insoluble radioactivity and protein content of these fractions was measured. In the relative specific activity (RSA) plots for rough- (RM) and smooth- (SM) microsomal fractions, the specific activity of the zero-time (0°) samples is set equal to one. The granule fraction (ZG) is normalized to the control chase condition. Data presented is the average of four experiments. Typical protein recoveries in RM, SM, and ZG were 0.5, 0.6, and 1.2 mg, respectively. At the end of the pulse the corresponding specific activities were 169,000, 104,000, and 15,000 cpm/mg protein.

μM A23187 in the presence of 1 mM EGTA and absence of calcium was without effect on intracellular transport.

Electron Microscopy

Since our previous studies of Ig secretion have shown the inhibitory action of monensin or A23187 in the absence of calcium to be associated with characteristic alterations of the Golgi complex (31), we have examined the ultrastructure of fibroblasts, macrophages, and the exocrine pancreas treated in a similar manner.

For both fibroblasts and macrophages, as for plasma cells (31), monensin treatment is accompanied by a disappearance of Golgi cisternae and the appearance of conspicuous dilated vacuoles in the Golgi region. These vacuoles often have a crenated perimeter, and, in the case of fibroblasts, a readily visible content (Fig. 7*a-d*).

Corresponding changes can be observed in monensin-treated pancreatic acinar cells. As in all the other cell types, stacked compressed Golgi cisternae are greatly reduced in number, and in their place are dilated vacuoles which are closely apposed, one to the next. Many of these vacuoles have a visible content which adheres to the inner aspect of their limiting membrane. This distribution of content is unlike that found in conventional condensing vacuoles in which such adherence is not common, (Fig. 8*a, b*). Condensing vacuoles are essentially absent after such monensin treatment.

Two other changes are frequently encountered in all three cell types after monensin treatment: transitional elements of the RER are often more abundant than in controls, and the mitochondria are condensed. Apart from these specific alterations, the cell ultrastructure is normal.

The treatment with A23187 in the absence of calcium (inhibitory only for fibroblasts) is accompanied by an increase in the population of Golgi vesicles in the case of fibroblasts (data not shown); but at the time point studied this increase is less striking than for plasma cells (31). Macrophages and acinar cells of the pancreas treated under the same conditions look entirely normal.

Autoradiographic Studies

As an independent means of exploring the site of monensin action in the exocrine pancreas, electron-microscopic autoradiography has been employed. Lobules were pulse labeled 5 min with [^3H]leucine and either fixed at once, chased one h

in control medium, or chased in the presence of 1 μM monensin. The results of such an experiment are given in Table I. At the end of the pulse, 91% of the autoradiographic grains are found associated with the RER. After the control chase they drain largely to Golgi elements and condensing vacuoles, and after chase in the presence of monensin they concentrate over dilated Golgi elements (condensing vacuoles are no longer present). Images characteristic of the chase conditions are given as Fig. 9.

DISCUSSION

Available evidence is consistent with the existence of a unique cytoplasmic pathway for secretory proteins of eukaryotic cells (24). After biosynthesis on the attached polysomes of the RER and segregation within its cisternal space, the proteins attain the partly-rough-partly-smooth transitional elements of the RER which give rise by membrane fission to smooth-surfaced vesicles. Such vesicles, along with associated smooth-surfaced cisternae, constitute the Golgi complex.

In cells that lack short-term control of secretory rate ("nonregulated" secretory cells) Golgi-derived vesicles are presumed to pass to the cell surface where they fuse with the plasma membrane and release their content by exocytosis. In these cases, it has not been evident what role the Golgi cisternae perform, though it has been established that they may contain secretory protein (e.g., plasma cells that synthesize anti-peroxidase antibodies [17]). In "regulated" secretory cells the Golgi elements give rise to immature condensing vacuoles that convert to storage granules or zymogen granules. In several cases (bovine pancreas [15], granulocytes [1, 21], lacrimal gland [11]), secretory product can be demonstrated in Golgi cisternae, although in the case of the guinea pig pancreas it has been suggested that vesicles may pass directly from transitional elements to condensing vacuoles (12). In the present study, we seek to identify conditions which perturb this traffic of smooth-surfaced elements to analyze its function, both in "regulated" and "nonregulated" secretory cells.

As a first step, we have sought to block secretion by several nonregulated secretory cells and thus to develop a "physiological profile" for protein discharge from such cells. In this paper we add to the information already obtained with normal and malignant plasma cells data concerning activated peritoneal murine macrophages and

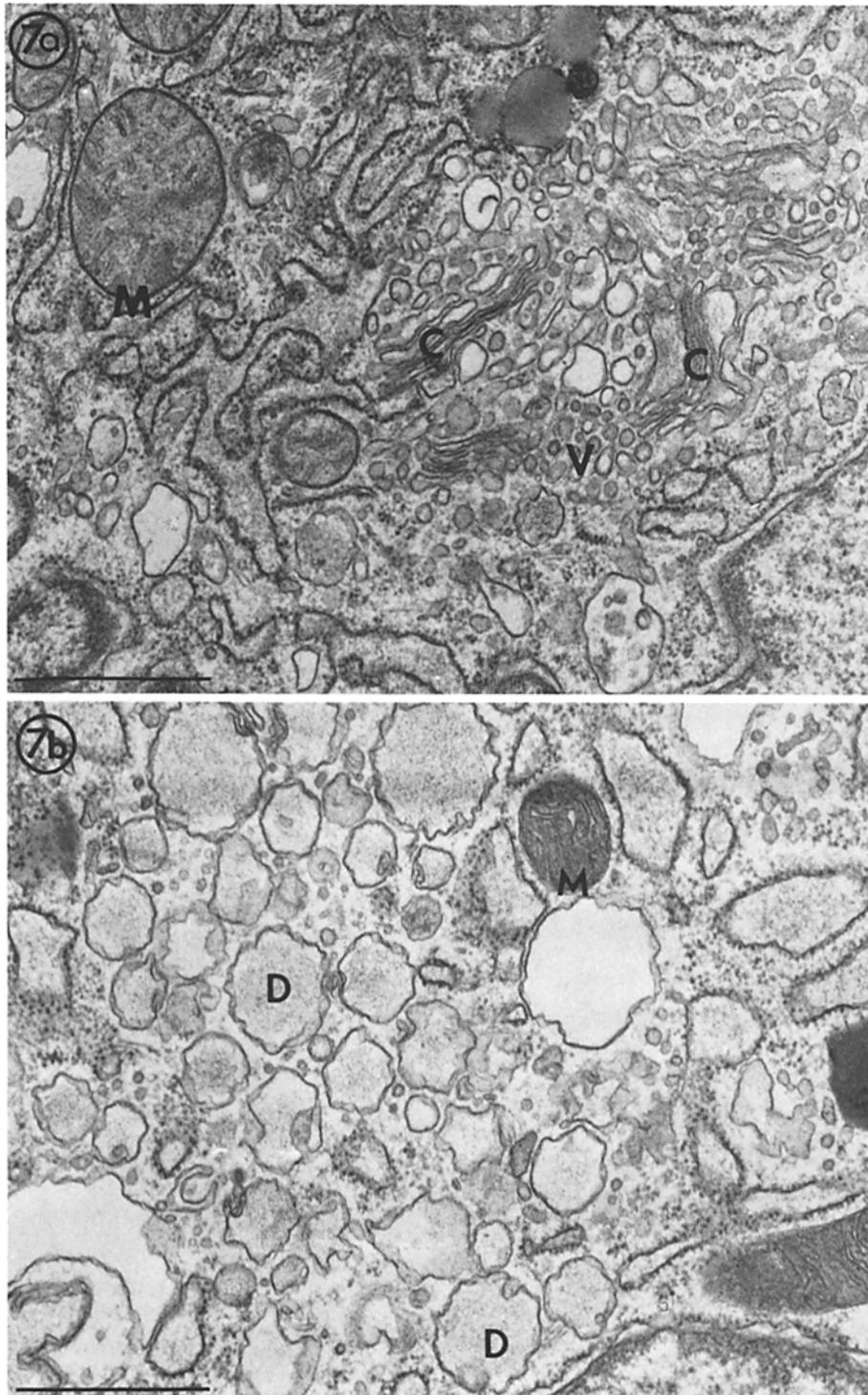
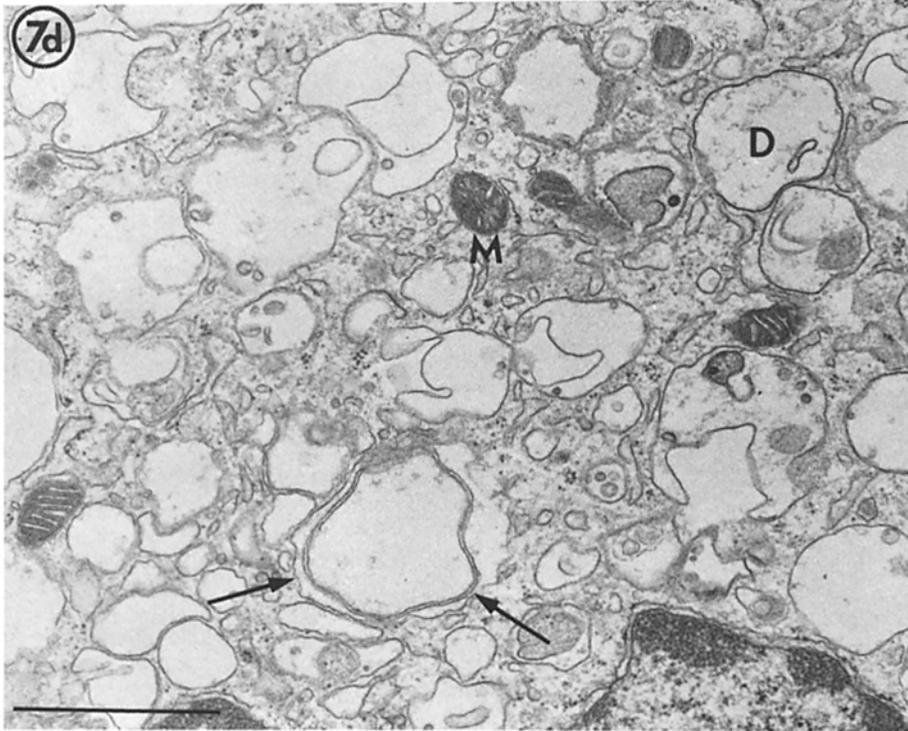


FIGURE 7 Ultrastructure of fibroblasts and macrophages after 1 h incubation at 37°C. (a) fibroblast control, (b) plus 1 μ M monensin, (c) macrophage control, (d) plus 1 μ M monensin. In (b) and (d) note the numerous dilated vacuoles in the Golgi region. Their content is especially evident in (b). In (d) the mutual orientation of the vacuoles is reminiscent of that of control Golgi cisternae e.g., in (c). C: Golgi cisternae; D: dilated vacuoles; M: mitochondria; V: Golgi vesicles. $\times 28,000$.



chick tendon fibroblasts. The comparative results are summarized in Table II. In all cases, discharge at maximal rates requires ongoing energy production and fails to be slowed by elimination of extracellular calcium or accelerated by attempted membrane depolarization with KCl. By contrast, depletion of cytoplasmic calcium (by use of the carboxylic ionophore A23187 in medium which lacks calcium and is supplemented with EGTA) is inhibitory for plasma cells and fibroblasts, though not for macrophages, for unknown reasons. The Na/K specific ionophore, monensin, is inhibitory for all three cell types.

The energy dependence observed can be partly

ascribed to the energy requirement for the exit of secretory protein from the RER (31); however, these may perfectly well be supplementary, more distal energy-requiring steps in the case of the nonregulated secretory cells. The dependence on cytoplasmic calcium presumably corresponds to a block within the Golgi region (31), yet the lack of apparent dependence in macrophages (and pancreatic cells) is puzzling and clearly limits the generality of such treatment with A23187. It is possible that such refractory cells have a means of selectively retaining an essential calcium pool which spares the secretory pathway from A23187 sensitivity.

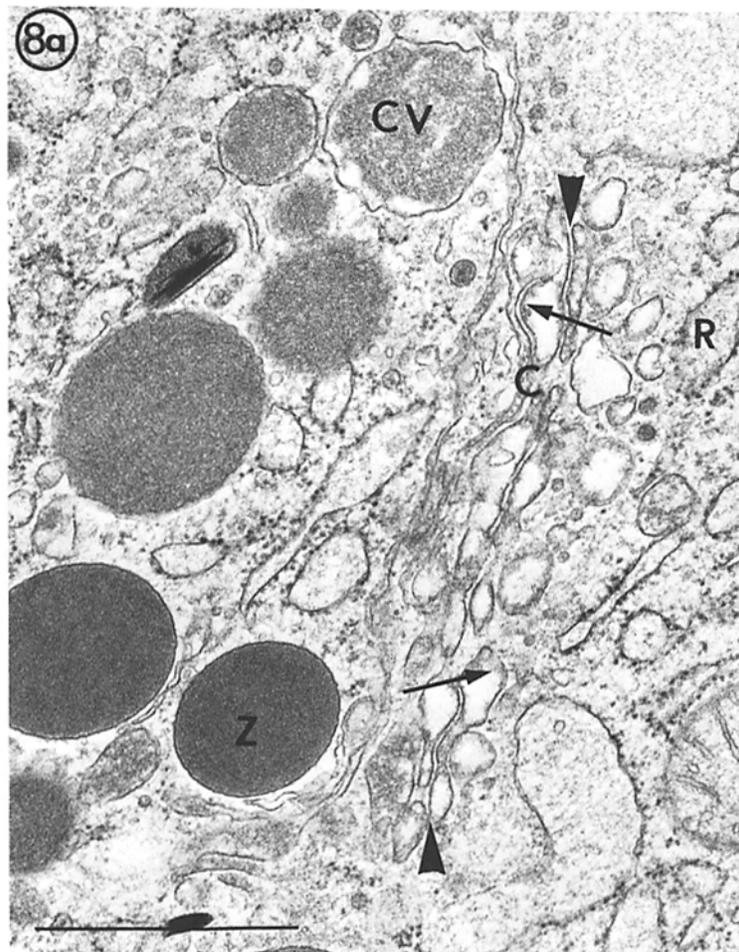


FIGURE 8 Ultrastructure of the exocrine pancreatic acinar cell. (a) control, (b), (c) after 1 μ M monensin, 45 min, 37°C. Note the conspicuous content lining the inner aspect of the limiting membrane of both compressed cisternae (a) and dilated vacuoles (b, c), long arrows. Short arrows indicate points of close apposition of adjacent vacuoles or cisternae. CV: condensing vacuoles; R: RER; Z: zymogen granules. Other abbreviations as above. (a) $\times 35,000$. (b) $\times 22,000$. (c) $\times 55,000$

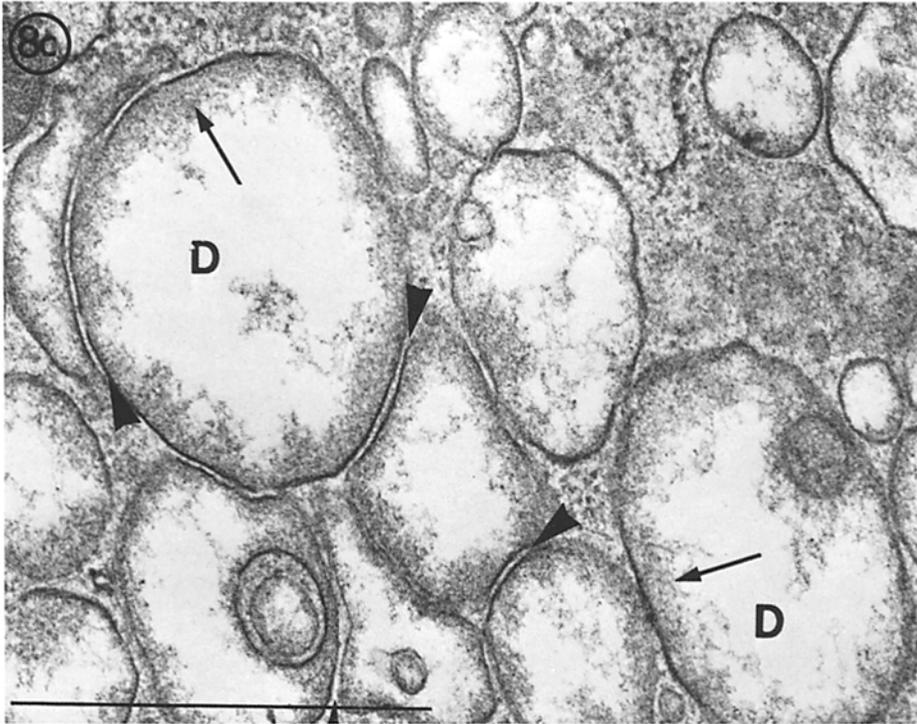
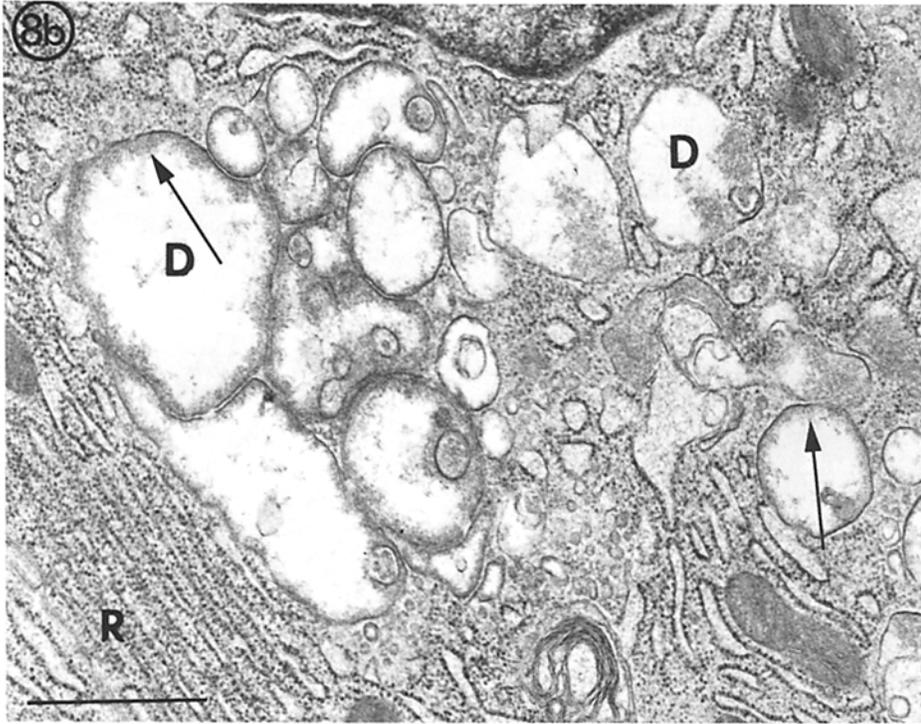


TABLE I
Distribution of Autoradiographic Grains

	RER	Golgi	Condensing vacuoles	Zymogen granules	Other	Grains counted
End of pulse, %	91	5	1	3	0	1,165
Control chase, %	26	33	35	2	3	295
Monensin chase, %	27	71	—	1	0	1,129

Pancreatic lobules were pulse-labeled 5 min with [^3H]leucine and then either fixed at once or first washed and reincubated 1 h with or without micromolar monensin in 5 $\mu\text{g/ml}$ cycloheximide-containing medium. After preparation for autoradiography, low-power electron micrographs were taken at random. A circle was drawn which circumscribed each autoradiographic grain, and the grain was assigned to the subcellular structure underlying the center of the circle.

For comparison, the corresponding physiological profile of a typical regulated secretory cell—the acinar cell of the exocrine pancreas—is also given in Table II. In so far as the discharge event itself is concerned, the two classes of secretory cell differ with respect to need for extracellular calcium, response to excess KCl, and the ionophore, monensin. These differences all pertain to the existence of a storage compartment, yet in all the cells studied (including those of the exocrine pancreas) monensin has a common and specific effect on intracellular transport at the level of the Golgi complex.

As already discussed in our studies of plasma cells (31), monensin causes dilation and possibly fusion of Golgi elements. We ascribe these events to the cytoplasmic ionic changes documented to occur in plasma cells; however, a further explanation is needed which accounts for the unique sensitivity of the Golgi complex and indicates why arrest of intracellular transport should be accompanied by such dilation or fusion. With regard to these questions, selected Golgi elements may contain Na or K-specific ion pumps which normally produce water efflux, although biochemical studies of isolated Golgi fractions have not yet revealed such activities. Furthermore, there may be ion-sensitive sites (enzymes?) which normally regulate membrane fusion and fission, but predominantly allow fusion to occur under the monensin-imposed high Na, low K conditions.

In the case of the macrophage and the pancreas, the origin of the monensin-induced vacuoles is belied by their persistent association with each other, much like the apposed compressed Golgi cisternae of control cells. Moreover, in the pancreas, one can distinguish between the monensin-induced vacuoles and Golgi cisternae, on the one hand, and condensing vacuoles, on the other. Only the former have a content which regularly

lines the inner aspect of their limiting membrane. One may judge from these morphological characteristics that the monensin-induced structures derive an important contribution from pre-existing Golgi cisternae; however, a proof of this relation will require histochemical (22) and (or) biochemical studies.

The present studies of the pancreatic secretory cycle clearly emphasize the specificity of monensin action, for in its presence drainage of secretory protein from the RER (Fig. 6a, Table I) and carbamylcholine-stimulated discharge (Fig. 5a) are not affected—only the vesicular traffic in the Golgi region is interrupted. No other agents are known to act specifically at this level except, possibly, for lanthanum (3) and colchicine (24, 26). Because protein synthesis continues in the presence of monensin, a storage compartment is generated in the Golgi region, but it is not possible to judge whether the apparently precipitated content of such monensin-induced vacuoles is actually insoluble *in vivo*.

Despite the condensation of mitochondria, the observations that protein synthesis and the early steps of intracellular transport continue in the presence of monensin, and that deliberate uncoupling of respiration does not cause Golgi vacuolization (14, 31), indicates that the blocks caused by monensin and CCCP are distinct.

It is of interest that a vacuolization of the Golgi area of other secretory cells has been correlated with K loss induced by hypoxia or triggering of membrane receptors; however, such studies have not explored a possible influence on intracellular transport (2, 29).

One sufficient unifying explanation of monensin's interruption of vesicular traffic is given in Fig. 10. We propose that the vesicles which originate from the transitional elements of the RER obligatorily fuse with Golgi cisternae, and

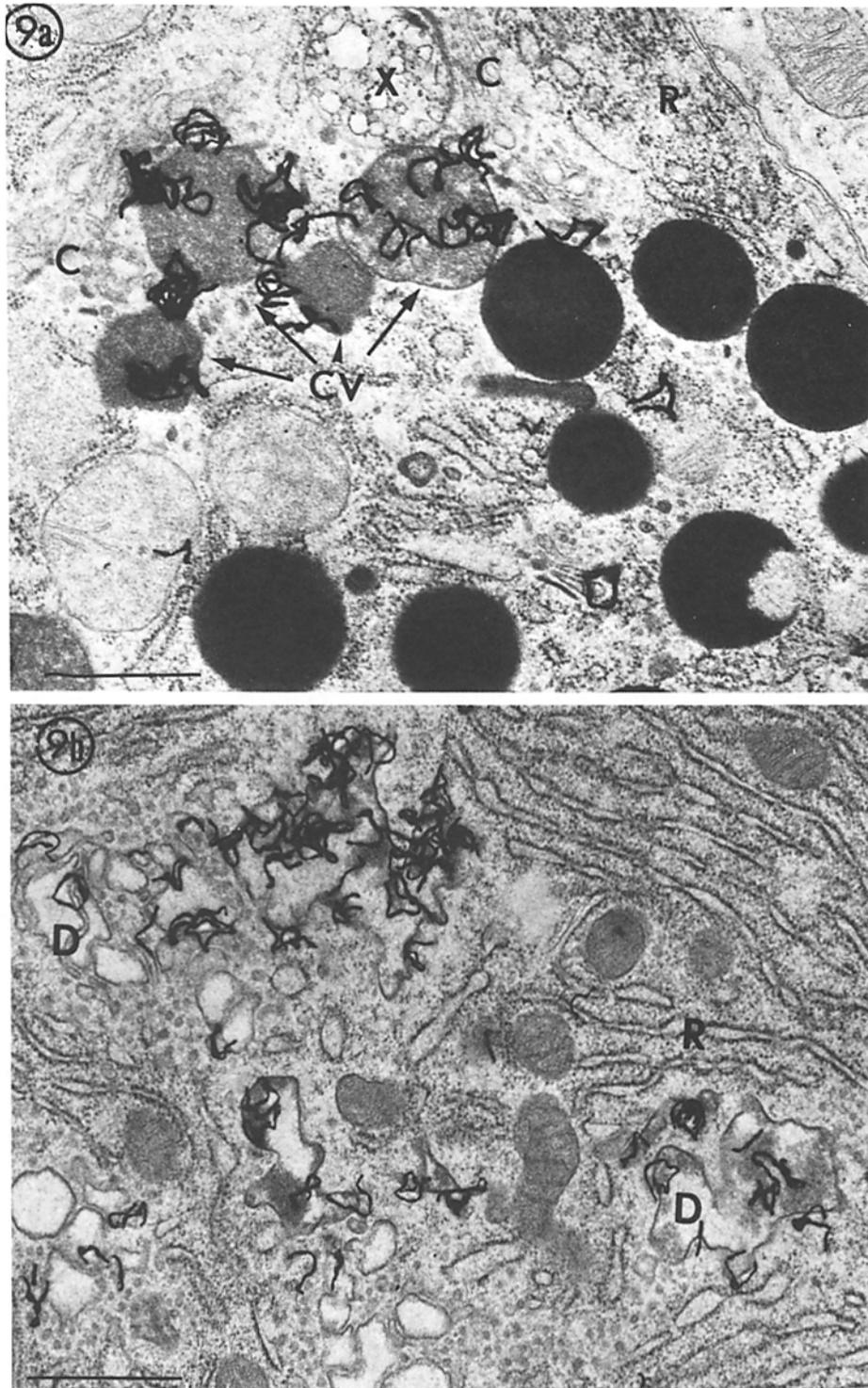


FIGURE 9 Electron microscopic autoradiography of the exocrine pancreas. (a) 5 min [^3H]leucine pulse followed by 1 h chase, (b) 5 min pulse followed by 1 h chase in the presence of 1 μM monensin. Note the accumulation of grains over condensing vacuoles (a) and dilated Golgi elements (b). Abbreviations as above.

TABLE II
Physiological Profile of Protein Discharge by
Secretory Cells

	Regulated		Nonregulated	
	Pancreas	Plasma cell	Macro-phage	Fibro-blast
Extracellular Ca^{++} requirement	+	-	-	-
Cyclic nucleotides implicated	+	-	?	?
Stimulated by extracellular K^+	+	-	-	-
Energy requirement	+	+	+	+
Influenced by cytoplasmic Na/K (monensin)	-	+	+	+
Intracellular Ca^{++} requirement (A23187 + EGTA)	+	+	-	+

The data summarized pertain to measurement of the release of biosynthetically labeled secretion product under short-term in vitro conditions. By the nature of such pulse-chase experiments, release is from zymogen granules for the regulated cell-type (exocrine pancreas) and for the other cell-types release is from Golgi-associated pools with a contribution from the RER. The data concerning plasma cells come from (31) and the data from pancreatic cells are reviewed in (31). All other data are from the present study.

that a secondary population of smooth-surfaced organelles derive from the cisternae and pass toward the cell surface. In nonregulated secretory cells that second population of organelles corresponds to small Golgi-derived vesicles endowed with a specificity which allows them to fuse with the plasma membrane. In regulated secretory cells, the second population corresponds to condensing vacuoles which "mature" to become storage granules. We propose that in all cases the effect of monensin is to arrest the membrane fission which creates such secondary organelle populations. Consequently, secretory product accumulates within Golgi cisternae. Earlier studies have identified the indicated sites of action of CCCP (14, 31).

Taken as a whole, the present data suggest that the Golgi complex, and in particular its cisternae, serve as a crossroad for the sorting out of smooth-surfaced membrane systems of the cell. They receive input from transitional elements (and perhaps from other sources), and export at least one distinct population of organelles, which is destined to participate in exocytosis. Previous biochemical

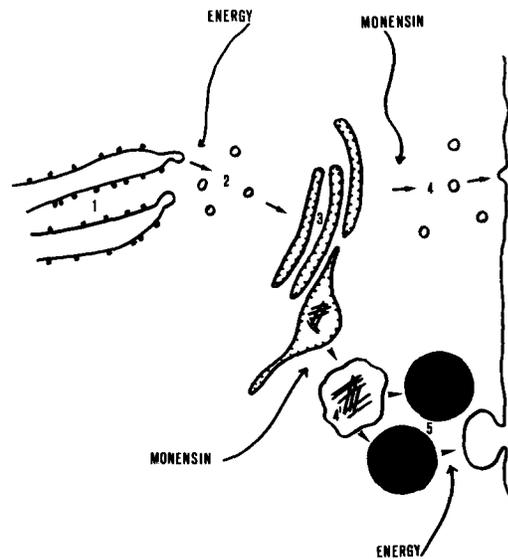


FIGURE 10 Model of intracellular transport pathways in regulated (lower) and nonregulated (upper) secretory cells, indicating the site of the proposed block imposed by monensin and CCCP. The structures of interest are, 1: RER, 2: primary Golgi vesicles derived from transitional elements, 3: Golgi cisternae, 4: secondary Golgi vesicles which pass to the cell surface in nonregulated secretory cells, 4': condensing vacuoles, 5: zymogen granules.

studies support such a concept in the sense that the enzyme composition of Golgi membranes, bearing small amounts of microsomal activities and plasma membrane activities, is itself distinct (8, 19). Similar observations have been made with respect to lipid composition (20).

The use of monensin may facilitate the routine isolation of Golgi subfractions, much as ethanol intoxication aids in isolation of the liver Golgi complex (7). The routine availability of such subcellular fractions would make possible studies of the processing of secretory product (e.g., proteolytic cleavage events, sugar addition, etc.), more precise identification of the generally inconspicuous intracellular pathway of secretory proteins in nonregulated secretory cells, and further elucidation of questions of membrane fusion, fission, and intermixing.

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REFERENCES

1. BAINTON, D. F., and M. G. FARQUHAR. 1970. Segregation and packaging of granule enzymes in eosinophil leukocytes. *J. Cell Biol.* 45:54-73.

2. BATZRI, S., Z. SELINGER, M. SCHRAMM, and M. ROBINOVITCH. 1973. K^+ release mediated by the epinephrine receptor in rat parotid slices. *J. Biol. Chem.* **248**:361-368.
3. BIEGER, W., J. SEYBOLD, and H. F. KERN. 1975. Studies on intracellular transport of secretory proteins in the rat exocrine pancreas. III. *Virchows Arch. A Pathol. Anat. Histol.* **368**:329-345.
4. BONNER, W. M., and R. A. LASKEY. 1974. A film detection method for tritium-labeled proteins and nucleic acids in polyacrylamide gels. *Eur. J. Biochem.* **46**:83-88.
5. COHN, Z. A., and B. BENSON. 1965. The differentiation of mononuclear phagocytes. Morphology, cytochemistry, and biochemistry. *J. Exp. Med.* **121**:153-170.
6. DEHM, P., and D. J. PROCKOP. 1971. Synthesis and extrusion of collagen by freshly isolated cells from chick embryo tendon. *Biochim. Biophys. Acta.* **240**:358-369.
7. EHRENREICH, J., J. J. M. BERGERON, P. SIEKEVITZ, and G. E. PALADE. 1973. Golgi fractions prepared from rat liver homogenates I. Isolation procedure and morphological characterization. *J. Cell Biol.* **59**:45-72.
8. EHRENREICH, J., J. J. M. BERGERON, P. SIEKEVITZ, and G. E. PALADE. 1973. Golgi fractions prepared from rat liver homogenates II. Biochemical characterization. *J. Cell Biol.* **59**:73-88.
9. EIMERL, S., N. SAVON, D. HEICHAL, and Z. SELINGER. 1974. Induction of enzyme secretion in rat pancreatic slices using the ionophore A23187 and calcium. *J. Biol. Chem.* **249**:3991-3993.
10. GORDON, S., J. TODD, and Z. A. COHN. 1974. In vitro synthesis and secretion of lysozyme by mononuclear phagocytes. *J. Exp. Med.* **139**:1228-1248.
11. HAND, A. R., and C. OLIVER. 1977. Relationship between the Golgi apparatus, GERL, and secretion granules in acinar cells of the rat exorbital lacrimal gland. *J. Cell Biol.* **74**:399-413.
12. JAMIESON, J. D. 1972. Transport and discharge of exportable proteins in pancreatic exocrine cells: *in vitro* studies. *Curr. Top. Membranes Transp.* **2**:273-338.
13. JAMIESON, J. D., and G. E. PALADE. 1967. Intracellular transport of secretory proteins in the pancreatic exocrine cell II. Transport to condensing vacuoles and zymogen granules. *J. Cell Biol.* **34**:597-615.
14. JAMIESON, J. D., and G. E. PALADE. 1968. Intracellular transport of secretory proteins in the pancreatic exocrine cell IV. Metabolic requirements. *J. Cell Biol.* **39**:589-603.
15. KRAEHNBUHL, J. P., L. RACINE, and J. D. JAMIESON. 1977. Immunocytochemical localization of secretory proteins in bovine pancreatic exocrine cells. *J. Cell Biol.* **72**:406-423.
16. LAEMMLI, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)* **227**:680-685.
17. LEDUC, E. H., S. AVRAMEAS, and M. BOUTELLE. 1968. Ultrastructural localization of antibody in differentiating plasma cells. *J. Exp. Med.* **127**:109-118.
18. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265-275.
19. MELDOLESI, J., and D. COVA. 1972. Composition of cellular membranes in the pancreas of the guinea pig IV. Polyacrylamide gel electrophoresis and amino acid composition of membrane proteins. *J. Cell Biol.* **55**:1-18.
20. MELDOLESI, J., J. D. JAMIESON, and G. E. PALADE. 1971. Composition of cellular membranes in the pancreas of the guinea pig. *J. Cell Biol.* **49**:130-149.
21. NICHOLS, B. A., D. F. BAINTON, and M. G. FAROUHAR. 1971. Differentiation of monocytes. *J. Cell Biol.* **50**:498-515.
22. NOVIKOFF, A., M. MORI, N. QUINTANA, and A. YAM. 1977. Studies of the secretory process in the mammalian exocrine pancreas. I. The condensing vacuoles. *J. Cell Biol.* **75**:148-165.
23. OLSEN, B. R., H. P. HOFFMANN, and D. J. PROCKOP. 1976. Interchain disulfide bonds at the COOH-terminal end of procollagen synthesized by matrix-free cells from the chick embryonic tendon. *Arch. Biochem. Biophys.* **175**:341-350.
24. PALADE, G. E. 1975. Intracellular aspects of the process of protein synthesis. *Science (Wash. D.C.)* **189**:347-358.
25. PETERSON, O. 1974. Cell membrane permeability change: an important step in hormone action. *Experientia (Basel)* **30**:1105-1107.
26. PROCKOP, D. J., R. A. BERG, K. I. KIVIRIKKO, and J. VITTA. 1976. Intracellular steps in the biosynthesis of collagen. In *Biochemistry of Collagen*. G. N. Ramachandran and A. H. Reddi, editors. Plenum Publishing Corporation, N.Y. 163-273.
27. SCHEELE, G. A., and G. E. PALADE. 1975. Studies on the guinea pig pancreas parallel discharge of exocrine enzyme activities. *J. Biol. Chem.* **250**:2660-2670.
28. SCHEELE, G. A., G. E. PALADE, and A. M. TARTAKOFF. 1978. Cell fractionation studies on the guinea pig pancreas. Redistribution of exocrine proteins during tissue homogenization. *J. Cell Biol.* **78**:110-130.
29. TAPP, R. L. 1970. Anoxic and secretory vacuolization in the acinar cells of the pancreas. *Q. J. Exp. Physiol. Cogn. Med. Sci.* **55**:1-15.
30. TARTAKOFF, A. M., and J. D. JAMIESON. 1974. Subcellular fractionation of the pancreas. *Methods Enzymol.* **31**:41-59.
31. TARTAKOFF, A. M., and P. VASSALLI. 1977. Plasma cell immunoglobulin secretion. Arrest is accompanied by alterations of the Golgi complex. *J. Exp. Med.* **146**:1332-1345.
32. UNKELESS, J. C., S. GORDON, and E. REICH. 1974. Secretion of plasminogen activator by stimulated macrophages. *J. Exp. Med.* **139**:834-850.
33. WEBB, Z., and S. GORDON. 1975. Secretion of a specific collagenase by stimulated macrophages. *J. Exp. Med.* **142**:346-360.
34. WEBB, Z., and S. GORDON. 1975. Elastase secretion by stimulated macrophages. *J. Exp. Med.* **142**:361-377.