Microtubules and Protein Secretion in Rat Lacrimal Glands: Localization of Short-term Effects of Colchicine on the Secretory Process

S. BUSSON-MABILLOT, A.-M. CHAMBAUT-GUÉRIN, L. OVTRACHT, P. MULLER, and B. ROSSIGNOL

Centre de Cytologie Expérimentale, 94200 Ivry, and Université Pierre et Marie Curie, 75230 Paris, and Laboratoire de Biochimie des Transports Cellulaires, Institut de Biochimie, Université de Paris Sud, 91405 Orsay Cédex, France

ABSTRACT The pathway and kinetics of the secretory protein transport in rat lacrimal exorbital gland have been established by an in vitro time-course radioautographic study of pulse-labeled protein secretion.

The colchicine-sensitive steps have been localized by using the drug at various times with respect to the pulse labeling of proteins. Colchicine (10 μM) does not block any step of the secretory protein transport, but when introduced before the pulse it decreases the transfer of labeled proteins from the rough endoplasmic reticulum to the Golgi area, suppressing their temporary accumulation in the Golgi area before any alteration of this organelle is detectable. Moreover, colchicine inhibits protein release only from the secretory granules formed in its presence because the peroxidase discharge is diminished 1 h after colchicine addition, and the secretion of newly synthesized proteins is strongly inhibited only when colchicine is introduced before secretory granule formation.

Morphometric studies show that there is a great increase of secondary lysosomes, related to crinophagy, as early as 40–50 min after colchicine is added. However, changes in lysosomal enzymatic activities remained biochemically undetectable.

We conclude that: (*a*) the labile microtubular system does not seem indispensable for protein transport in the rough endoplasmic reticulum-Golgi area but may facilitate this step, perhaps by maintaining the spatial organization of this area; and (*b*) in the lacrimal gland, colchicine inhibits protein release not by acting on the steps of secretion following the secretory granule formation, but by acting chiefly on the steps preceding secretory granule formation, perhaps by making the secretory granules formed in its presence incapable of discharging their content.

From data obtained with various secretory tissues, it appears that the microtubular system could be involved in the secretory process (62). We have previously shown in rat lacrimal glands that colchicine inhibited the release of newly synthesized proteins by impairing their intracellular transport (9), and simultaneously induced a disorganization of the labile microtubular system. This latter effect could result from the shift of the equilibrium between microtubules and tubulin and from the impediment of microtubule assembly due to the colchicine–tubulin complex (10). The intracellular transport alterations produced by colchicine have also been observed in other tissues that produce exportable proteins (liver: 17, 52–55; pancreas B-cells: 36, 37; fibroblasts: 12, 14, 38; anterior pituitary: 1, 26, 48; mammary gland: 30, 41, 42; parotid gland: 46; exocrine pancreas: 61; parathyroid gland: 11, 29). Although there is no definite proof, the hypothesis that both events (i.e., inhibition of protein secretion and disruption of labile microtubules) might be related by a causal effect is favored.

The aim of the present work is to localize the colchicine-sensitive step(s), which could be microtubule-related, in the secretory process. We have thus established the pathway and kinetics of secretory protein migration in normal lacrimal glands and analyzed the short-term effect(s) of colchicine using both ultrastructural and biochemical approaches.

THE JOURNAL OF CELL BIOLOGY · VOLUME 95 OCTOBER 1982 105–117

MATERIALS AND METHODS

Rats were male albino Sprague-Dawley of ~150 g body weight, fed ad libitum. Lacrimal glands were dissected and incubated in Krebs-Ringer bicarbonate buffer as previously described (9).

Measurement of Protein Secretion

LABELING AND DISCHARGE OF PROTEINS: Proteins were pulse-labeled for 3.5 min by the incubation of lobules (130 mg) with $U^{14}C$-leucine (5–25 μCi). Incorporation was stopped by adding fresh buffer supplemented with 1 mM leucine and washing three times. Incubation was carried out as described previously (9) for 70 min. When the effect of colchicine was tested, the drug was introduced (10 μM final concentration) at various times with respect to the radioactive tracer addition, as indicated in the legends to figures. The lobules were then washed three times with a colchicine-free buffer and subjected to further incubation in a fresh medium, with or without 2 μM carbachol as an inducer of secretion.

EVALUATION OF PROTEIN SECRETION: At the times indicated under each figure, aliquots from the incubation medium were withdrawn and centrifuged. The lobules were blotted, weighed, then homogenized with an Ultra-Turrax homogenizer for 30 s at 4°C either in water (for labeled protein secretion measurement) or in 0.1 M Tris, pH 7.35 (for peroxidase activity measurement), and centrifuged at 37,000 *g* for 20 min at 4°C. The supernatant fractions were analyzed for their radioactive protein content as previously described (9) and for their peroxidase activity according to Putney et al. (51). One unit of peroxidase was defined as a change in absorbance at 460 nm of 1,000 in 1 min.

Secretion was expressed in either of two ways: (*a*) as the amount of labeled proteins or peroxidase activity released into the incubation medium per gram of tissue, or (*b*) as the amount of labeled proteins or peroxidase activity present in the medium, as a percentage of the total soluble labeled proteins or total peroxidase activity in tissue and medium (9). Both modes of expression gave the same pattern of secretion kinetics.

Lysosomal Enzyme Assays

β-*N*-acetylglucosaminidase activity was determined according to Sellinger et al. (60) on aliquots of the incubation medium and the 37,000 *g* supernatant from lobules homogenized in 0.05 M citrate buffer, pH 5.0, with 0.1% Triton X-100. Cathepsin D activity was measured, as described in reference 4, on aliquots of the incubation medium and on the 37,000 *g* supernatant from lobules homogenized in 0.25 M formiate buffer, pH 3.2, with 0.1% Triton X-100. Hemoglobin was used as a substrate.

Protein Analysis

^{14}C-labeled gland lobules were homogenized in Tris-sucrose (50–240 mM) buffer, pH 6.8 (50 mg of tissue/ml). Aliquots from the 37,000 *g* supernatant and from the centrifuged incubation medium were analyzed by Sephadex G-100 filtration for radioactivity, for protein content according to Lowry et al. (34), and for peroxidase activity. They were also analyzed by PAGE using the discontinuous system described by Laemmli (31) with 3% polyacrylamide stacking gel (pH 6.7) and 13% polyacrylamide resolving gel (pH 8.6). Proteins were fixed and stained in 0.05% Coomassie Blue R-250 in water-methanol-trichloroacetic acid (5:5:1, vol:vol:wt), and destained in water-methanol-trichloroacetic acid (38:2:3, vol:vol:wt). Standards were run under the same conditions. Bands were recorded using a Vernon microdensitometer (model PH I 6, Paris, France). Disk gels were analyzed for radioactivity as described previously (10). Slab gels were dried, then exposed to Kodak X-ray film for an appropriate time to detect the radioactive bands without overexposing. When the sample applied on the gel contained between 1,500 and 3,000 cpm, 195 h was found to be optimal. Detection of minor bands required longer exposure. Tracings of appropriately exposed films were made with a recording microdensitometer. Under these conditions the darkening was proportional to the radioactivity. Thus, the height of each peak from the recorded tracing was directly related to the radioactivity of the proteins from the corresponding band and was taken as a measure of radioactivity. Results are expressed either as the mean ± SEM or as a single representative experiment of the whole.

Electron Microscope Radioautography

Lobules were preincubated for 20 min in the absence or in the presence of 10 μM colchicine, then pulse-labeled for 3.5 min with ^{3}H-3,4,5-L-leucine (40 μCi/ml). Tritium incorporation was stopped by adding fresh buffer containing 1 mM leucine and then washing three times. After a chase period of 50 min and a washing period of 10 min, the lobules were incubated for 40 min with or without 2 μM carbachol as an inducer of secretion. When colchicine was present during the 20-min preincubation period, it was also added for the chase and washing periods. Lobule samples were collected at 0, 10, 30, 50, 80, and 100 min after the end of the pulse. Each time-sample was fixed with glutaraldehyde, postfixed with osmium tetroxide, and embedded in Araldite, as usual.

Thin sections were prepared for electron microscope radioautography according to the conventional dipping method (described in 6) using Ilford L4 emulsion and a Kodak Microdol developer. Measurements were made only on the outermost cells, at the periphery of the lobules, i.e., cells in direct contact with the incubation medium, with sections of completely visible cells (including lumina, nucleus, and basal area) being selected for analysis.

Two modes of quantitative calculation of radioactivity were used (see 13): (*a*) the radiation label density (RLD), i.e., the relative silver grain number over each compartment referred to the silver grain number over the whole cell (percentage):

$$\text{RLD} = \frac{\text{number of silver grains over a compartment}}{\text{number of silver grains over the cell}} \times 100;$$

(*b*) the specific-radiation label density, i.e., the radiation label density of a given compartment referred to the relative surface of this compartment

$$\text{Relative surface} = \frac{\text{surface of a cell compartment}}{\text{surface of the cell}} \times 100.$$

Therefore:

$$\text{Specific-radiation label density} = \frac{\text{radiation label density}}{\text{relative surface}} \times 100.$$

The evaluation of developed silver grains and the morphometric study were made on the same photographic print. Each developed silver grain was attributed to one of the seven following cell compartments: nucleus, rough endoplasmic reticulum, Golgi area (saccules and small vesicles), condensing vesicles, secretory granules, lysosomes, or mitochondria (Fig. 1). Each developed silver grain was assigned to the cell compartment overlapped by the geometrical center of the tangle (40). For statistical reliability, about 900 silver grains were counted for each time point. The surface of the different cell compartments was determined by planimetry (MOP 1 Kontron, Kontron Messgeraete, Eching-Munchen) directly on micrographs (final enlargement: × 17,000), the profile of each organelle being previously traced out (Fig. 1).

Chemicals

L-[U-^{14}C]leucine, L-[^{3}H-3,4,5]leucine, D-[U-^{14}C]galactose, D-[U-^{14}C]glucose (respectively, 318 mCi/mmol, 30 Ci/mmol, 240 mCi/mmol, 350 mCi/mmol) were purchased from the Commissariat à l'Energie Atomique, Gif-Sur-Yvette, Cedex (C.E.A.). Colchicine was obtained from Calbiochem-Behring Corp., American Hoechst Corp., (San Diego, CA); carbamylcholine chloride (carbachol) from Mann Research Laboratories (New York); and *O*-nitrophenyl-*N*-acetyl-*p*-D-glucosaminide, 3-3′-diaminobenzidine tetrahydrochloride, and hemoglobin from Sigma Chemical Co. (St. Louis, MO). Sephadex G-100 was purchased from Pharmacia Fine Chemicals (Pharmacia le Chesnay, France); acrylamide, *N,N′*-methylenebisacrylamide, and Temed from Eastman Kodak Company (Rochester, NY). All other chemicals used were reagent grade.

RESULTS

Effect of Colchicine on Protein Release

The noninduced release of peroxidase (a secretory protein: 15, 24) (Fig. 2*a*) was not affected by colchicine during the first hour of incubation, but was decreased by it afterwards. In colchicine-treated lobules the carbachol-induced release of peroxidase (Fig. 2*a*) followed the same rate as in the control for the first 30 min after carbachol addition; thus, colchicine did not alter the cholinergic response. But, after 30 min, the induced peroxidase release had decreased, and after 60 min no more peroxidase appeared in the medium. The time course of the carbachol-induced release of pulse-labeled proteins that had migrated for 140 min followed the same kinetics as peroxidase release and underwent the same modifications as peroxidase release when colchicine was added.

Light and electron microscope observations (Fig. 3) showed that carbachol induced a depletion of secretory granules in acinar cells incubated under control conditions (Fig. 3*b*, and *e*, and reference 23), but not in colchicine-treated cells that still retained numerous secretory granules (Fig. 3*c* and *f*).

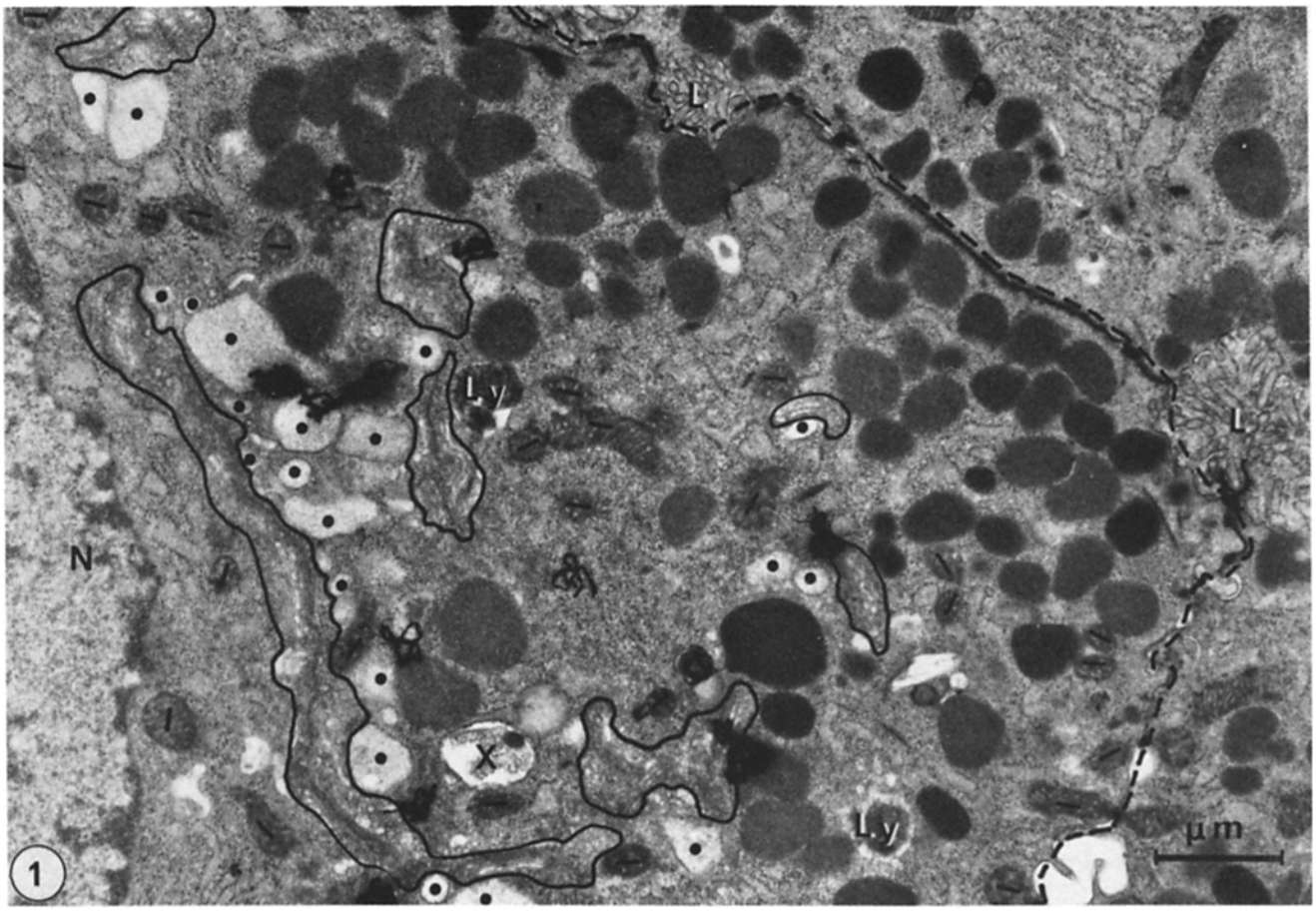


FIGURE 1 Example of micrography prepared for radioautographic and surface measurements. The structures to be measured are marked. Dashed line, cell contour; bars, mitochondria; full line, Golgi area; dot, condensing vesicle; *X*, autophagic vacuole. Nucleus (*N*) and dark secretory granules, being quite recognizable, were not delineated in advance. Note that the rough endoplasmic reticulum cisternae are scattered all over the cell, including the apical cytoplasm between secretory granules. *L*, lumen; *Ly*, secondary lysosome. Bar, 1 µm. × 17,000.

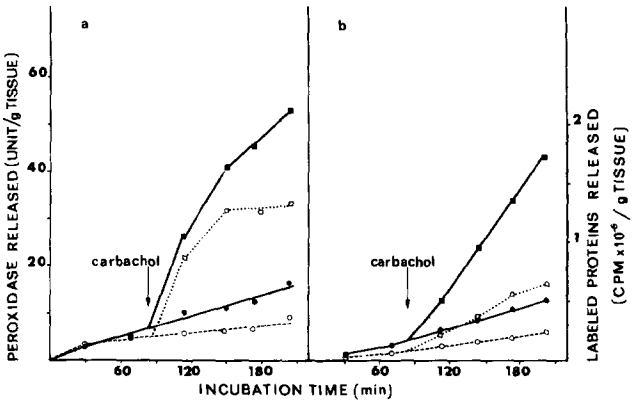
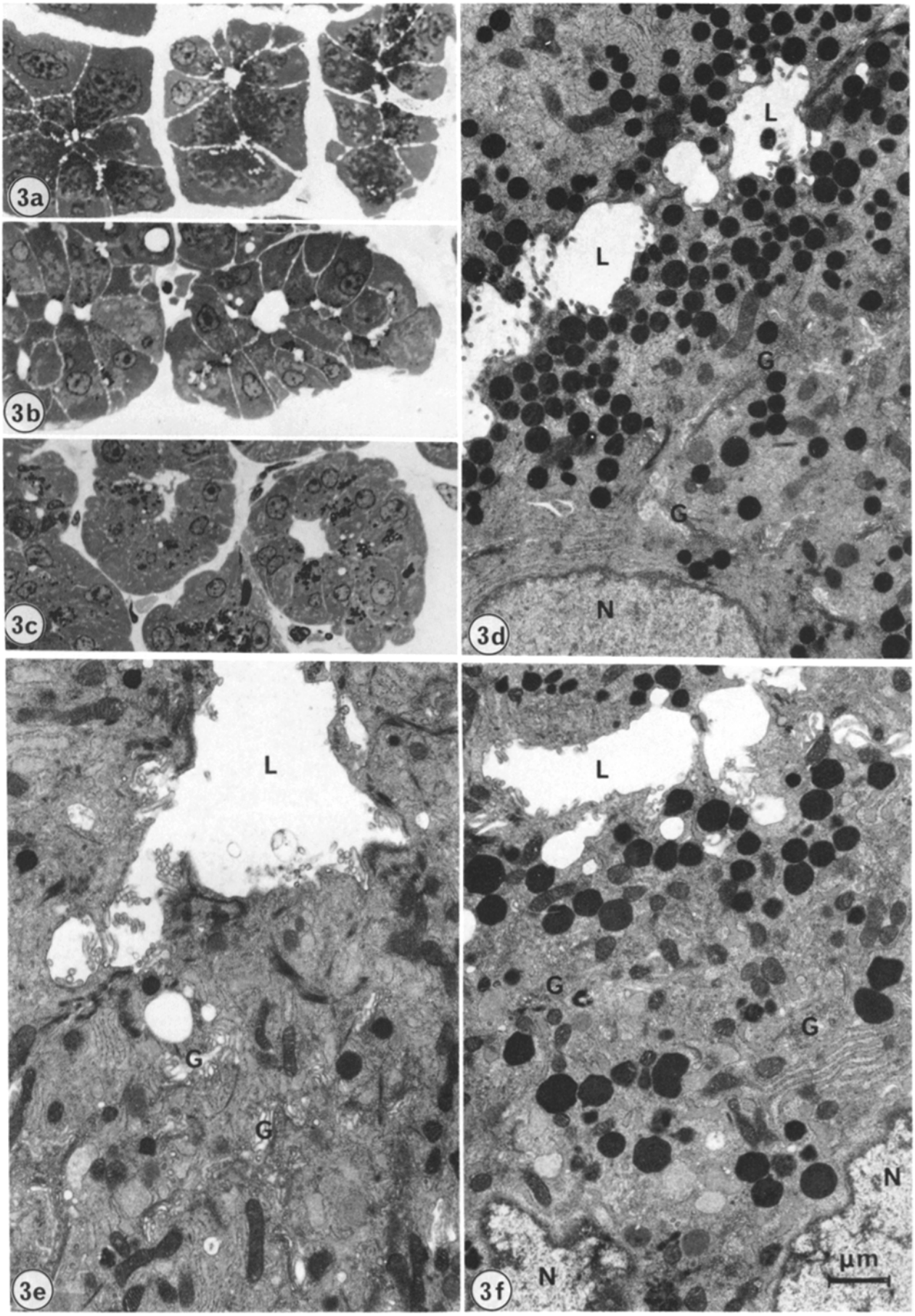


FIGURE 2 Influence of colchicine on noninduced and carbachol-induced protein release. (*a*) Influence on peroxidase release. (*b*) Influence on the release of newly labeled proteins synthesized in the presence of colchicine. Peroxidase activity and radioactive proteins were measured on aliquots withdrawn from the incubation medium every 30 min and on lobules collected at the end of the incubation period, as described in Materials and Methods. Data are expressed as cumulative release of peroxidase or labeled proteins into the incubation medium. The same results were obtained if the incubation medium was changed every 30 min. Noninduced release: ●, control, ○, colchicine; carbachol-induced release: ■ control, □, colchicine.

The ^{14}C-labeled proteins (i.e., newly synthesized) began to appear in the incubation medium 30 min after the onset of the labeling (Fig. 2*b*) and their release was immediately decreased by colchicine. The carbachol-induced release of neosynthesized ^{14}C-proteins (Fig. 2*b*) was inhibited with colchicine by ~70%, whatever the time period.

Together, the above results show that colchicine does not alter the release of already present peroxidase, thus leading to the conclusion that the peroxidase released after 1 h of incubation is newly synthesized because its release, like the release of newly synthesized ^{14}C-proteins, is sensitive to inhibition by colchicine.

To check whether colchicine could affect the secretion of a particular neosynthesized protein, we made a comparative analysis of gland extracts and incubation medium from control and colchicine-treated lobules by gel filtration (data not shown) and by gel electrophoresis (Fig. 4 and Table I). The radiolabeled proteins, which were released in roughly constant proportions under carbachol stimulation (bands d to h in Table I), were also released in constant proportions in colchicine-treated lacrimal lobules, but in lesser amounts (Fig. 4 and Table I). In this case, each individual protein was affected by the same percentage of release inhibition (75% inhibition of the noninduced or carbachol-induced secretion). Table I also shows that after carbachol stimulation more secretory proteins were re-


3a
3b
3c
3d
L
L
G
G
N
3e
L
G
G
3f
L
G
G
N
N
µm

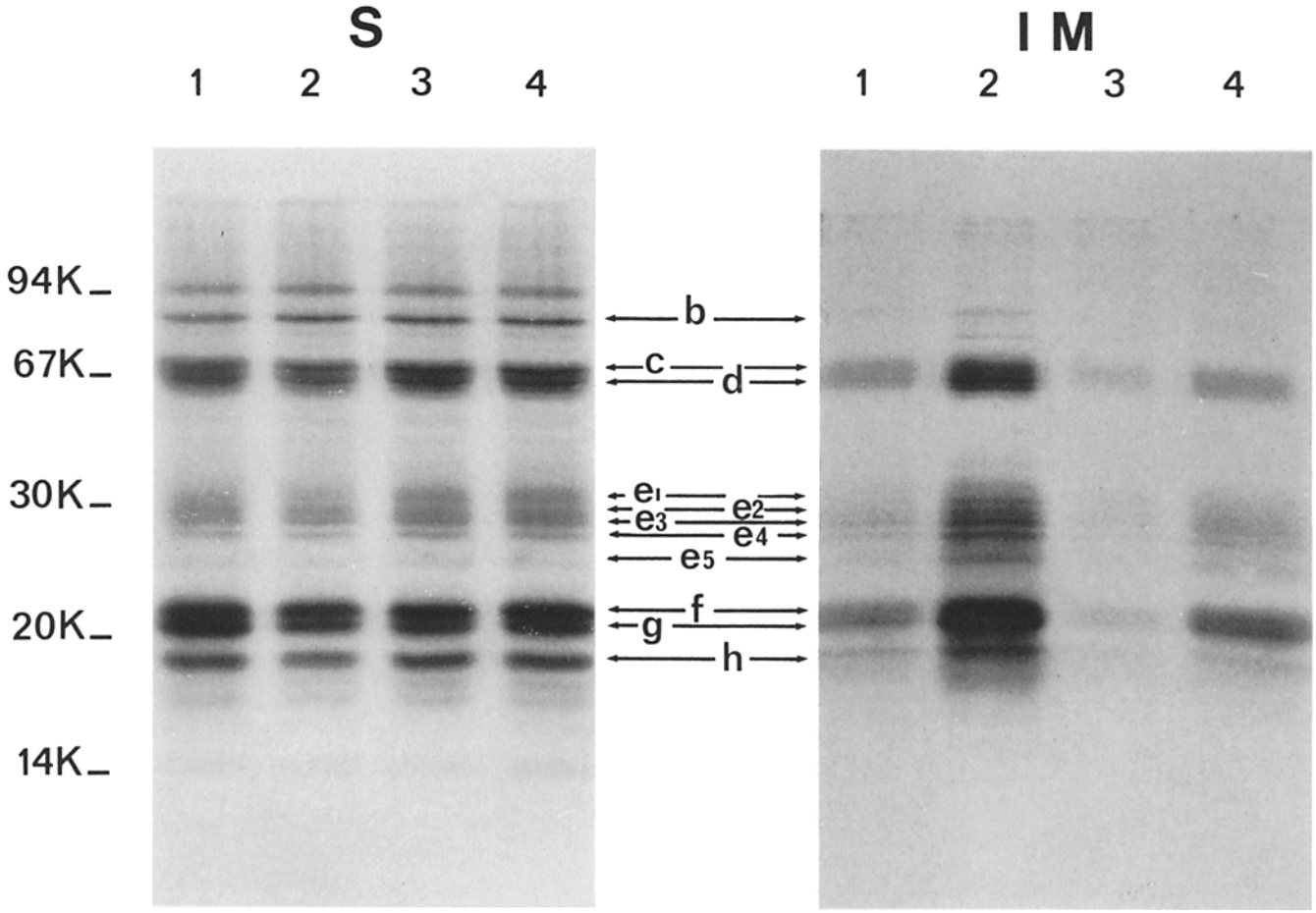


FIGURE 4 Influence of colchicine on the [^{14}C]leucine labeled protein composition of lacrimal glands and on the composition of the labeled proteins released into the incubation medium, under noninduced or carbachol-induced conditions. Lobules were labeled for 75 min with 25 μCi of [^{14}C]leucine, with or without colchicine, then washed and reincubated in a colchicine-free medium for 60 min, with or without carbachol. Aliquots from the incubation medium (*IM*), and from the gland supernant (*S*) fractions were prepared and electrophoresized in the absence of mercaptoethanol as described in Materials and Methods. Assay *1*: control; *2*: carbachol; *3*: colchicine; *4*: colchicine and carbachol. Aliquots from soluble gland extracts corresponded to 0.8 mg fresh weight of glands and aliquots from incubation medium corresponded to 0.5% of the initial volume, i.e., to the labeled protein released in 1 h by 0.65 mg of glands. The gels were exposed for radioautography for 330 h (*S*) and 500 h (*IM*). Arrows and numbers indicate the molecular weight scale determined on the same gel stained with Coomassie Brilliant Blue. The main labeled bands were named as letters indicated in front. In this experiment the following secretions were found to be: *1*: 14.7%; *2*: 45.8% (carbachol effect: +212%); *3*: 7.2% (colchicine effect: −51%); *4*: 16.2% (carbachol effect: +123%; colchicine effect: −65%).

tained in colchicine-treated lobules than in untreated ones (compare lines *S2* and *S4*). Thus, a high percentage of new secretory proteins formed in the presence of the drug could not be released.

Degree of Inhibition of Protein Release as a Function of the Moment of Colchicine Addition

Fig. 5 shows the noninduced secretion of proteins labeled with ^{14}C-leucine when colchicine was added at various times during the incubation: before pulse, at the onset of the pulse, and after the end of the pulse period. Part *a* depicts an example of the kinetics. It appears (part *b*) that the degree of inhibition depends on the time when colchicine was added with respect to the radioactive tracer introduction: 75% inhibition if colchicine was present from 15 to 30 min before pulse; 55% at the onset; 40% when colchicine was added 10 min after the pulse. When it was added later, the inhibition progressively decreased to zero after 55 min. The degree of inhibition of the carbachol-induced release also depended on the time of colchicine addi-

FIGURE 3 Effect of carbachol on control and colchicine-treated cells. Light micrographs show the general aspect of several acini submitted to the following treatments. (*a*) Control lobules incubated in Krebs-Ringer buffer alone. (*b*) Lobules incubated first in Krebs-Ringer, then submitted to carbachol. (*c*) Lobules treated with colchicine and then submitted to carbachol. Electron micrographs show the cell apices in the same samples, as follows: (*d*) control; (*e*) Krebs-Ringer followed by carbachol; (*f*) colchicine followed by carbachol. In the controls (*d*), cell apices are filled with dark secretory granules. Carbachol obviously induces the secretory granule discharge in cells incubated in Krebs-Ringer buffer alone (*e*) but has a markedly reduced effect on colchicine-treated cells (*f*) since they retain many clusters of secretory granules. *G*, Golgi area. *L*, lumen. *N*, nucleus. *a*, *d*: Krebs-Ringer, 100 min post pulse; *b*, *e*: Krebs-Ringer 60 min post pulse plus carbachol 40 min; *c*, *f*: colchicine 60 min plus carbachol 40 min. Bar, 1 μm. *a*, *b*, *c*: × 600; *d*, *e*, *f*: × 11,000.

TABLE I

Electrophoretic Analysis of the Labeled Proteins Released into the Incubation Medium and from Gland Supernatant Fraction. Influence of Colchicine and of Carbachol

Band		b	c	d	e_1	e_2	e_3	e_4	e_5	f	g	h
IM1.	Control	5.8	24.0	25.5	12.0	15.5	17.5	15.0	10.0	43.0	48.0	22.0
IM2.	Carbachol	12.0	89.0	106.0	55.0	69.0	85.4	81.0	55.0	180.0	222.0	89.5
	Carbachol effect(2/1)%	*107*	*275*	*316*	*358*	*345*	*389*	*440*	*450*	*320*	*362*	*307*
IM3.	Colchicine	2.5	10.0	7.0	5.0	7.2	8.2	7.2	4.5	14.5	14.2	9.0
	Colchicine effect(3/1)%	*−57*	*−58*	*−79*	*−78*	*−77*	*−76*	*−75*	*−72*	*−83*	*−85*	*−78*
IM4.	Colchicine and carbachol	5.5	24.0	24.5	12.5	21.0	22.5	19.0	13.5	56.0	60.5	19.5
	Carbachol effect(4/3)%	*120*	*140*	*250*	*150*	*190*	*175*	*165*	*200*	*285*	*325*	*120*
	Colchicine effect(4/2)%	*−54*	*−73*	*−77*	*−77*	*−70*	*−74*	*−77*	*−76*	*−69*	*−73*	*−78*
	Colchicine effect upon carbachol stimulation(%)	*12*	*−49*	*−21*	*−58*	*−45*	*−52*	*−63*	*−56*	*−10*	*−10*	*−62*
S2.	Carbachol	31.8	29.0	26.2	11.0	14.0	14.0	10.0	5.0	46.5	41.0	23.5
S4.	Colchicine and carbachol	33.5	41.5	38.5	17.2	19.0	20.0	13.5	6.0	65.0	53.0	33.2

Polyacrylamide gel obtained from the experiment described in the legend to Fig. 4 was exposed for radioautography for 195 h for gland supernatant (*S*) and for 500 h for incubation medium (*IM*) definition of radioactive bands. The radioactivity from each band (b to h as indicated in Fig. 4) was directly related to the peak height of the recorded tracing (see Materials and Methods). Roman type represents peak height in millimeters. Italic type indicates either the stimulatory effect of carbachol or the inhibitory effect of colchicine.

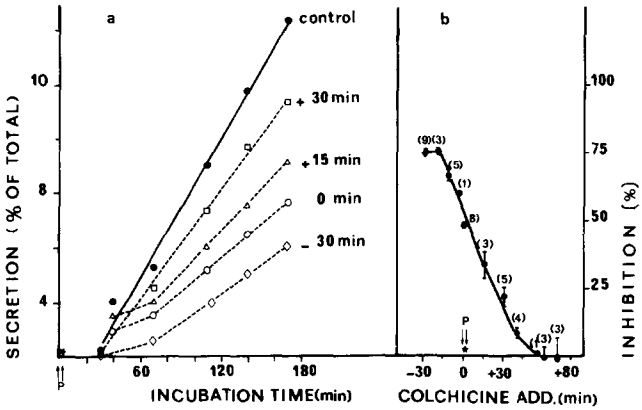


FIGURE 5 Effect of colchicine on newly synthesized [^{14}C]leucine labeled protein release. Influence of the time of colchicine addition relative to the pulse period. Colchicine was added 30 min before the pulse (◇), at the onset (○), or at various times after the end of the pulse period (△, 15 min; □, 30 min; ● refers to control, without colchicine treatment). Arrows indicate the pulse (*) period. After a migratory period of 70 min for labeled proteins, the incubation medium was replaced with fresh buffer and incubation pursued. At each time indicated, all of the incubation medium (5 ml) was removed and analyzed for ^{14}C-labeled proteins and was replaced with fresh buffer. When colchicine was added 40 min or more after the end of the pulse, it was also present during the discharge period. (*a*) Results are expressed as the percentage of protein secreted from the onset of incubation to the time considered (cumulative discharge) to total protein (total protein released during the whole incubation, plus gland supernatant). (*b*) Results are expressed as the percentage of inhibitory effect produced by colchicine on secretion, as a function of the moment of colchicine addition relative to the onset of the pulse period. Mean ± SEM. Numbers in brackets refer to the number of experiments.

tion with respect to the pulse and varied as did that of the noninduced release: 70% when the drug was added 30 min before the pulse; 62% at the onset; 50%, 30%, and 9% when it was introduced 10, 30, or 70 min, respectively, after the pulse.

Analysis of Cholchicine Short-term Effects on Each Step of the Secretory Pathway

PROTEIN BIOSYNTHESIS

Total peroxidase activity level (gland extracts plus incubation medium), which was kept roughly stable during a 3-h incubation in the control, was not modified in the continuous presence of colchicine. In the same way, colchicine had no effect upon [^{14}C]leucine or [^{14}C]galactose incorporation into total proteins under the conditions used throughout the present work. Neither [^{14}C]leucine incorporation into the individual proteins of the supernatant fraction nor the relative percentage of each protein were altered by colchicine, at least in an obvious way (Fig. 4 and Table I).

SECRETORY PROTEIN INTRACELLULAR TRANSPORT DETERMINED BY QUANTITATIVE RADIOAUTOGRAPHY

The lobules were incubated for 20 min with colchicine (10 μM) before pulse-labeling with [^{3}H]leucine in order to start the secretory cycle of newly synthesized proteins under conditions where the labile microtubules were recovered as colchicine-tubulin complex (9). We found that, under our conditions, the previously formed colchicine-tubulin complex remained stable throughout the experiment, after colchicine removal from the incubation medium, and that protein release was inhibited by ~75%.

ROUGH ENDOPLASMIC RETICULUM PLUS SURROUNDING HYALOPLASMIC MATRIX: These two compartments could not be separated in grain assignment, as rough endoplasmic reticulum (*rER*) cisternae were too closely apposed in lacrimal gland cells (Fig. 1). Thus, the results are given simultaneously for these two compartments and referred to as *rER-H* (rough Endoplasmic Reticulum-Hyaloplasm).

In colchicine-treated lobules, the rER-H-radiation label density (Fig. 6) was quite similar, but with two obvious modifications. First, between 0 and 50 min post pulse, the rER-H-radiation label decrease was delayed (~20 min), and second, the remnant radioactivity was slightly higher and this value was maintained till the end of the experiment.

GOLGI AREA: In control lobules the Golgi area specific-radiation label density (Fig. 7) abruptly increased and reached a maximum at 50 min post pulse (that corresponding to a 100% increase). Concentration then rapidly decreased, but remained at a relatively high level from 80 min post pulse to the end of the experiment (100 min after pulse). When lobules were incubated with colchicine, the radioactivity reached and then left the Golgi area, as in controls, but obviously, the concentration peak disappeared (Fig. 7).

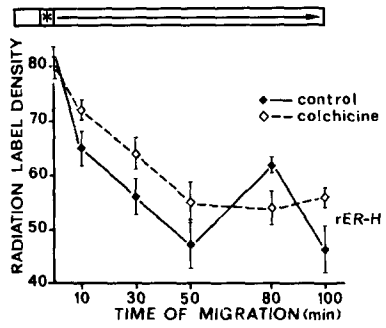


FIGURE 6 Evolution of the *rER-H* (rough Endoplasmic Reticulum-Hyaloplasm) radiation label density in control and colchicine-treated lobules. This mode of expression gives the relative grain count in a compartment (i.e., without taking into account the volume of this compartment). Bars indicate SEM. Colchicine is added 20 min before pulse (*) and is present in the medium during labeled protein migration (arrow).

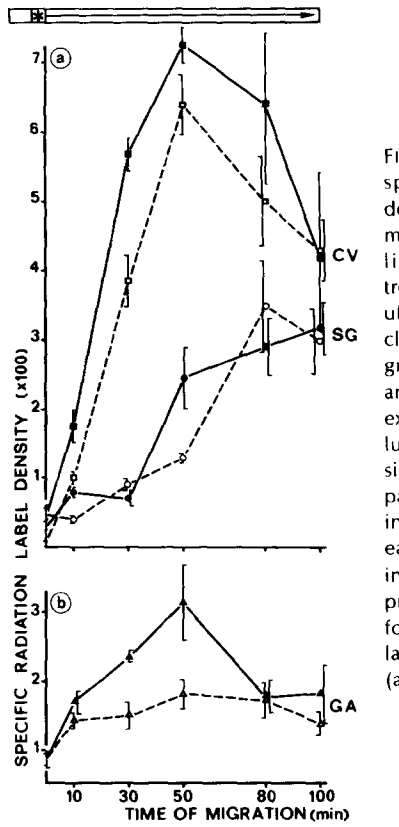


FIGURE 7 Evolution of specific-radiation label density in cell compartments of control (solid lines) and colchicine-treated (dashed lines) lobules. (a) Condensing vesicles (*CV*) and Secretory granules (*SG*), (*b*) golgi area (*GA*). This mode of expression gives the absolute number of developed silver grains in each compartment (i.e., by taking into account the surface of each compartment). Bars indicate SEM. Colchicine is present in the medium before pulse (*) and during labeled protein migration (arrow).

CONDENSING VESICLES AND SECRETORY GRANULES: In controls the concentration of label in condensing vesicles increased nearly 17 times between 0 and 50 min post pulse and then markedly decreased, whereas the concentration of label in secretory granules began to increase only 30 min after pulse[1] and was still increasing at the end of the experiment (Fig. 7). In the presence of colchicine, condensing vesicle radioactivity evolution was quite similar but slightly delayed and did not reach as high a level as in the control. In the same way, after the first 30 min, radioactivity in secretory granules began a gradual increase and at 80 min even exceeded the control level. After 80 min the radioactivity decreased in secretory granules (Fig. 7), in contrast to the control.

[1] In the first 30 min, the number of silver grains over the secretory granule compartment was too low to give a significant value for specific-radiation label density.

Effects of Colchicine on the Structure of Cell Compartments Involved in the Secretory Process

Short incubation with colchicine, i.e., 120 min total incubation time, did not produce extensive modifications in the cell organization, but the effects of colchicine on cell structure were constant.

The regular organization of rER cisternae was not disturbed, but after 20 min of incubation with colchicine, small vesicles and short saccules were already visible at the basal pole. These structures, which will be referred to as Golgi-apparatuslike (GA-like) formations, presented the following characteristics: light content (Fig. 8*a*), close association with rER cisternae (Fig. 8*c*) and mitochondria, and, in longer incubation times, large dark granules that became heterogeneous after 60 min (Fig. 8*d*) and were similar to lysosomes. In control cells these GA-like formations were never observed, even after 120 min of incubation (Fig. 8*b*).

At the beginning of incubation, as in the controls, the Golgi apparatus consisted of stacked, elongated saccules extending across the whole cell above the nucleus (Fig. 9*a* and *b*). After a 70-min incubation with colchicine some stacks were markedly shortened, swollen, and surrounded by numerous small vesicles (Figs. 9*c* and 10*a* and *b*). In this 120-min experiment the Golgi-area relative surface presented only variations around a mean value of 6.7%, and when incubated with colchicine did not show any obvious decrease before 70 min. In the same way, condensing vesicle surface, which represents between 1 and 3% of the total cell surface, was not significantly impaired by colchicine.

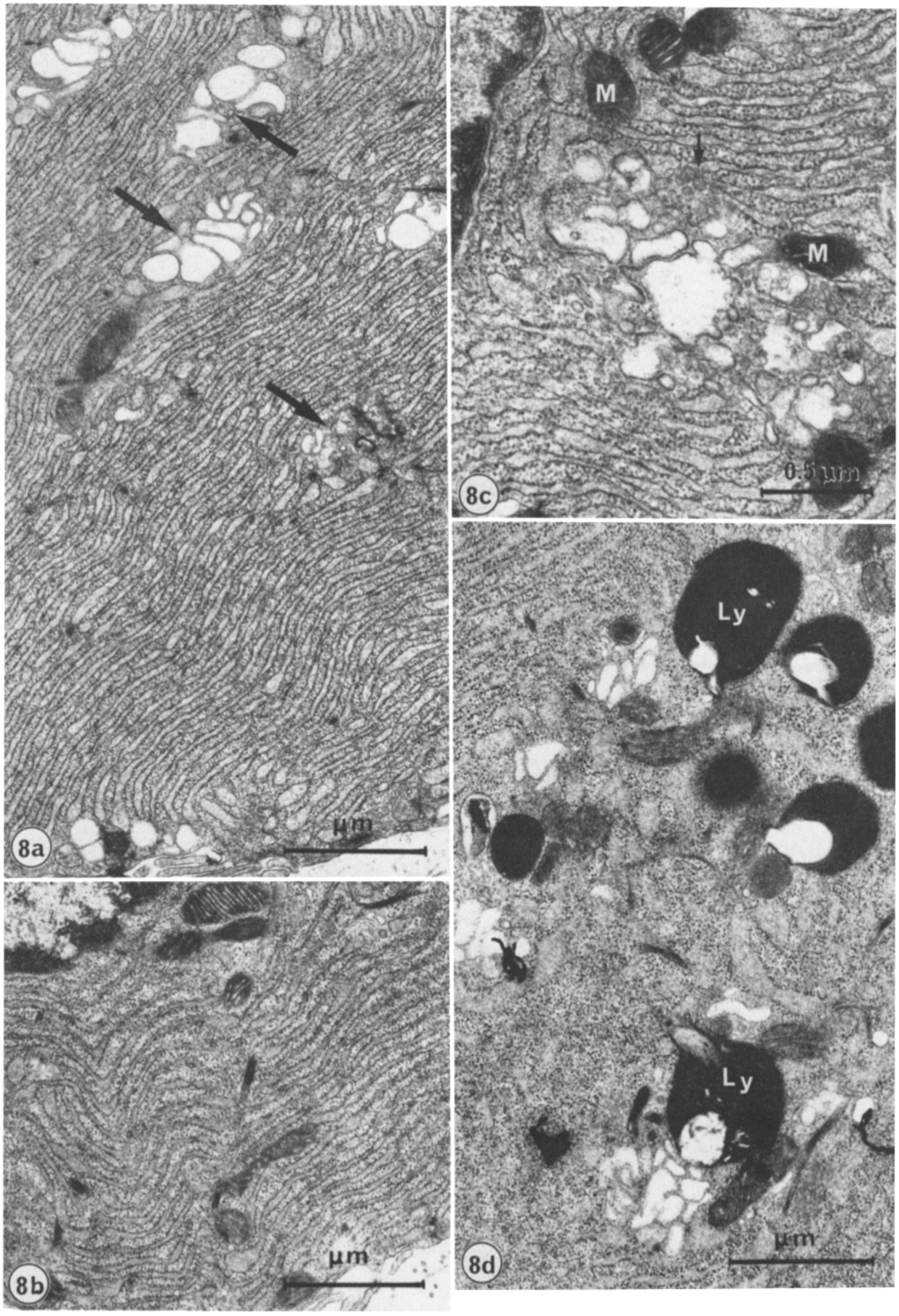
The secretory granules almost completely filled the cell apex in the control (Fig. 3*a* and *d*), whereas after 100 min of incubation with colchicine they formed well separated clusters, usually close to the Golgi area (Fig. 3*c* and *f*).

Colchicine and Lysosomal Activity

Colchicine very quickly evoked in acinar cells a strong increase in secondary lysosomes, as evaluated by surface measurements. The secondary lysosome relative surface remained small, ~0.5% of the total cell surface and quite similar in both sets of lobules, up to 30 min of incubation. Then it increased strikingly (to 2%) in lobules incubated with colchicine and stayed at this high level during the whole incubation (Fig. 11). Meanwhile, secondary lysosome relative surface in control lobules remained constant, ~0.5%.

In colchicine-treated lobules, these lysosomes appeared simultaneously very close to both the Golgi area and the basal GA-like formations. In the Golgi area, from 30 min of colchicine incubation onwards, some secretory granules showed small clear spots in their dark content. Then, in the same location, besides these organelles, heterogeneous bodies (secondary lysosomes) became numerous (Fig. 10). These bodies were always surrounded by a single membrane and never contained recognizable cell organelles (e.g., ribosomes or mitochondria).

Lysosome activity was evaluated by radioautography, by determination of β-*N*-acetyl-glucosaminidase and cathepsin D activities, and by protein analysis. In colchicine-treated cells, lysosome radioactive labeling was very low and irregular between 20 and 100 min of total incubation time, but after 120 min nearly every lysosome was labeled. Under the same conditions, no significant variation of β-*N*-acetylglucosaminidase and cathepsin D activities was detected between 100 and 190


8a
µm
8b
µm
M
M
0.5 µm
8c
Ly
Ly
µm
8d

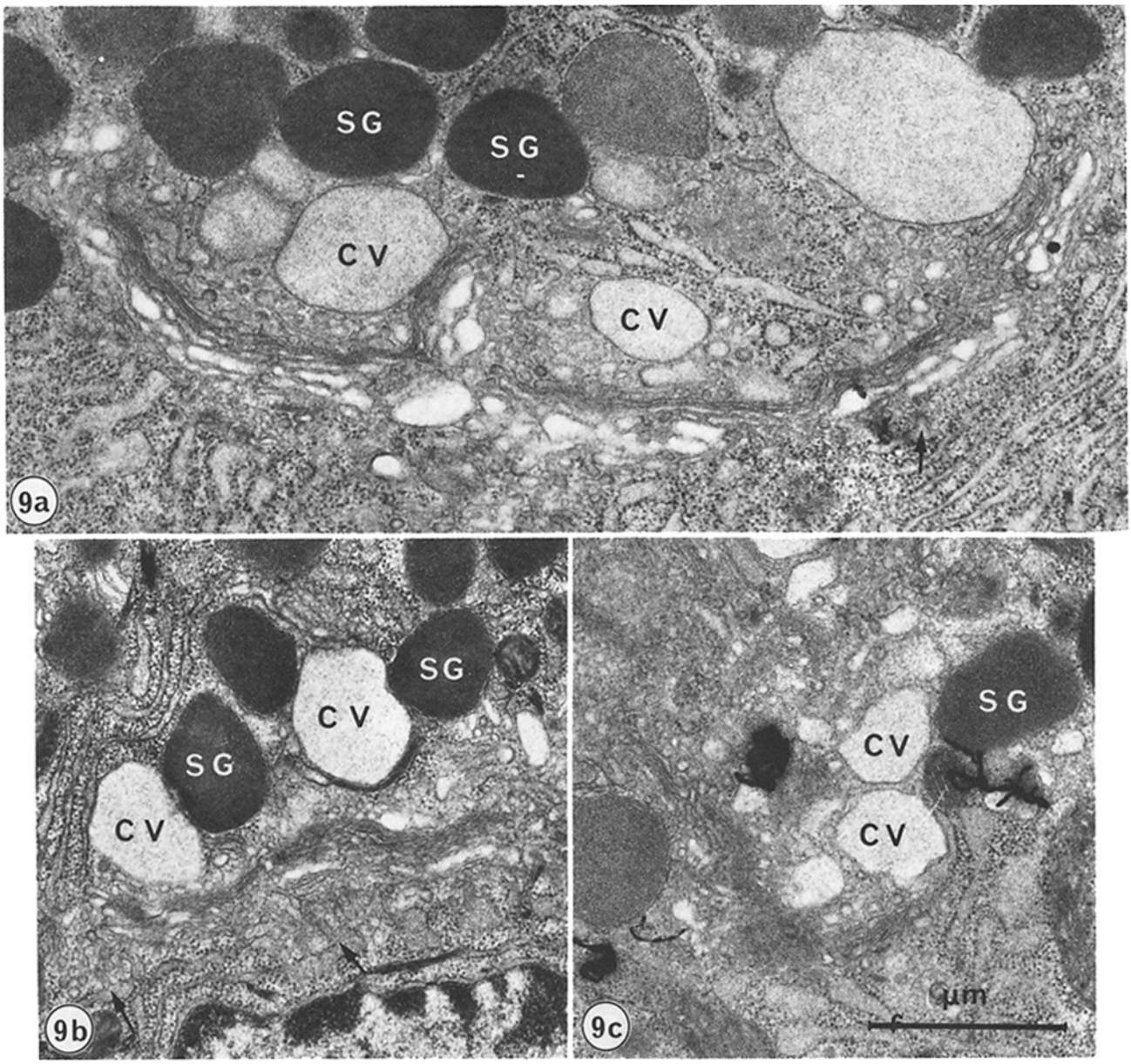


FIGURE 9 Golgi area of lacrimal gland cells. In controls, the Golgi area contains stacked elongated saccules surrounded by small vesicles, from the onset of incubation (*a*) to the end of incubation (*b*: 120-min incubation). Small arrows indicate rER-blebs visible at the forming face of the Golgi stack. After a 70-min incubation with colchicine (*c*) some Golgi stacks are markedly reduced and surrounded by more numerous vesicles. *CV*, condensing vesicle; *SG*, secretory granule. Bar, 1 μm. × 31,000.

min. However, when the glands were incubated for 3 h in the continuous presence of colchicine, they displayed an enhanced activity of β-*N*-acetylglucosaminidase compared with the control (+ 20%). The analysis by Sephadex G-100 filtration and gel electrophoresis of proteins labeled with [^{14}C]leucine or [^{14}C]glucose did not show any obvious degradation of cell proteins induced by colchicine for a 2-h period, whether colchicine was added before, during, or after the labeling.

DISCUSSION

Normal Intracellular Transport of Secretory Proteins

Evaluation of specific-radiation label density (Fig. 7) shows that labeled proteins reach the Golgi area and associated condensing vesicles 10 min after pulse, and appear in the secretory granules from 30 to 50 min onwards. They are to be

FIGURE 8 Basal area of lacrimal gland cells. GA-like formations (large arrows) are visible between the rER cisternae in colchicine-treated cells (*a*) but not in controls (*b*: 120-min incubation in Krebs-Ringer buffer). These GA-like formations are close to mitochondria (*M*; *c*) and surrounded by small vesicles and rER blebs (small arrows) that give to these formations their Golgi apparatus appearance. For longer incubation time (*d*) secondary lysosomes (*Ly*) are regularly associated with the GA-like formations. Bar, 1 μm. *a*, *b*, *d*: × 26,000; *c*: × 40,000.

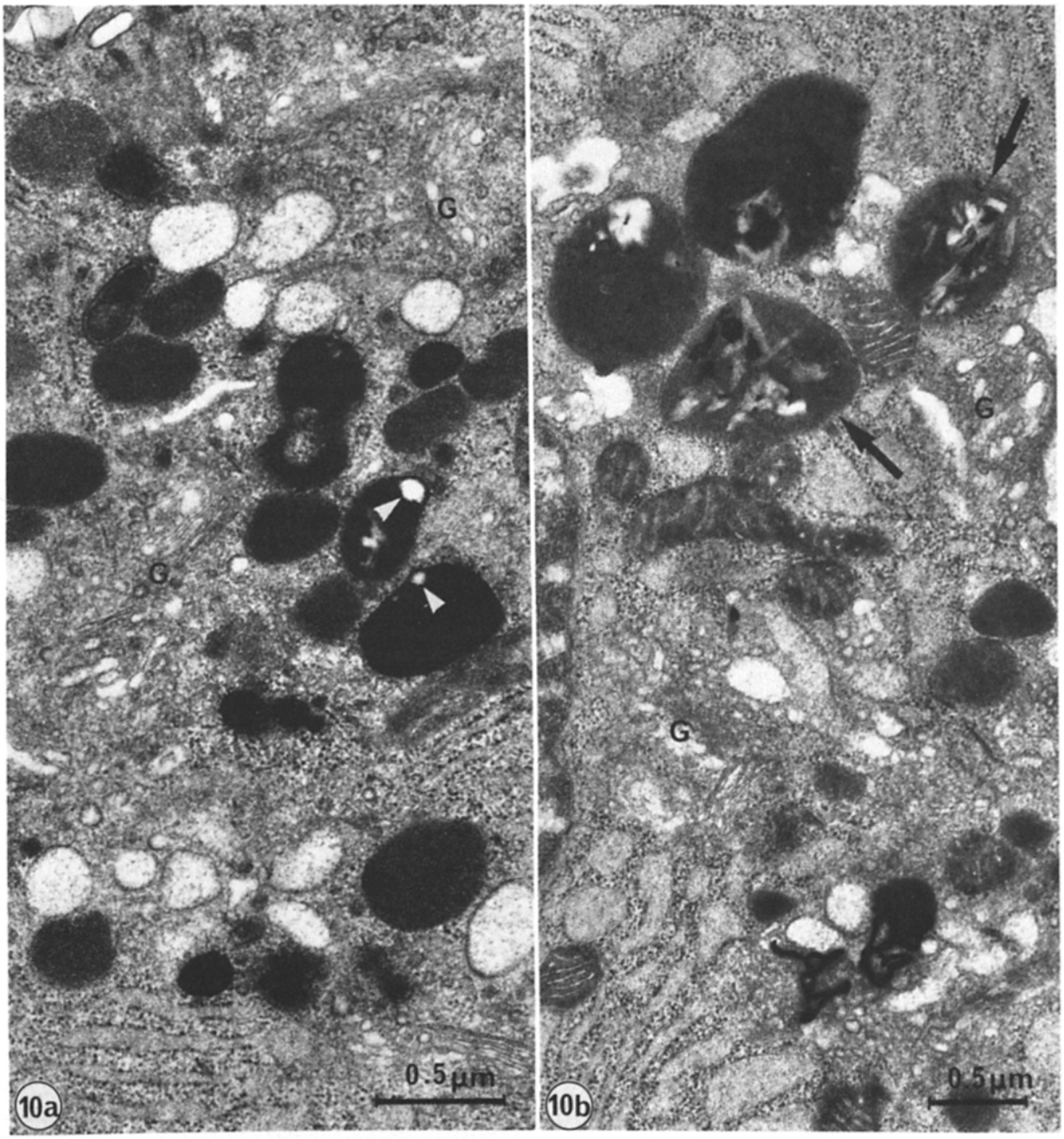


FIGURE 10 Evolution of dark granules in Golgi area of colchicine-treated cells: two states observed in 100-min-treated cells. These granules first (*a*) present clear spots (arrowheads) in their homogeneous content, then they enlarge (*b*) and become markedly heterogeneous (large arrows) like secondary lysosomes. *G*: Golgi area. Bars, 0.5 µm. *a*: × 40,000; *b*: × 31,000.

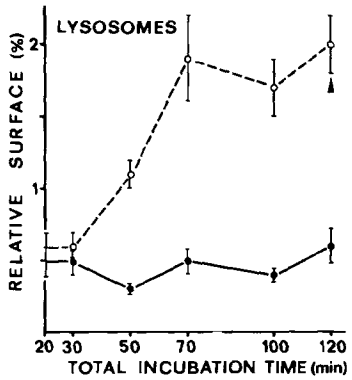


FIGURE 11 Relative surface of secondary lysosomes in control (●) and colchicine-treated (○) cells. Arrowhead indicates the time at which all lysosomes begin to be regularly labeled. Bars indicate SEM.

found in the incubation medium 30 min after the onset of labeling (Fig. 2), but continue to accumulate in secretory granules even 100 min after the pulse. Thus the secretory protein transport is achieved, according to the classic secretory pathway (45), at a rate comparable to that observed in various glands of mammals, such as: Brunner's gland (57), mammary gland (58), liver (2, 20), parotid gland (8), endocrine pancreas (44), and prostate (19). In all these glands, the proteins are still accumulating in the secretory granules 1 h after pulse. On the contrary, the whole secretory process is much faster in seminal vesicles (18), odontoblasts (68), and parathyroid (21), in which the radiation-label density in secretory granules is already decreasing 30 min after pulse.

The rough endoplasmic reticulum drainage seems to be incomplete in lacrimal glands because, after 1 h, the rER-H still contains 50% of its initial radioactivity, whereas it contains only 20–25% in most other glands, except the seminal vesicle and prostate (18, 19). The most likely interpretation of this result is that the remaining rER-H radioactivity represents labeled nonsecretory proteins in rER or hyaloplasm. Besides, the rER-H radioactivity shows a significant peak between 50 and 80 min in lacrimal glands as well as in parathyroid (21), and perhaps in other glands such as the following: exocrine (27) and endocrine (44) pancreas, Brunner's glands (57), and parotid gland (8). Habener et al. (21) believe that this secretory increase might represent "reutilization, in protein synthesis, of radioactive amino-acids derived from the cellular metabolism of radiolabeled proteins."

Colchicine Short-term Effects on Secretion

Our results indicate that colchicine affects the secretory process in two distinct ways: first, colchicine delays the intracellular transport of secretory proteins already in the rER (7), and second, besides this delay, colchicine inhibits the discharge of secretory granules formed in its presence.

DELAY OF SECRETORY PROTEIN INTRACELLULAR TRANSPORT: In our conditions, the first colchicine effect on secretion is revealed by two interrelated facts: the slight lag in rER drainage and the obvious suppression of the radioactivity peak in the Golgi area (Figs. 6 and 7). In untreated cells the radioactivity peak in the Golgi area clearly indicates a protein accumulation resulting from an excess of protein input, with respect to output, in the Golgi area. In colchicine-treated cells, the suppression of this peak is caused by a decrease of the protein input in the Golgi area because, first, the Golgi area volume is not modified at the time considered and, second, the protein output from the Golgi area remains unaltered as deduced from the specific-radiation label density of condensing vesicles. Thus, colchicine modifies the very onset of the secretory pathway by delaying the secretory protein transport from rER to Golgi area, and likely even earlier, i.e., in the rER. Similar results have been reported in mammary gland (42; 1 μM colchicine) and pancreas B-cells (37; 100 μM colchicine, for longer periods). Similarly, but under somewhat different conditions, the radioactivity peak in smooth microsomes was delayed (61; exocrine pancreas), and a lag occurred in the conversion of hormone or plasma protein precursors in parathyroid gland (11, 29), endocrine pancreas (35), chondrocytes (33), and hepatocytes (55). Moreover, in a few cases, accumulation of secretory molecules in the rER was detected after long incubations (17, 53, 61, 46).

As concerns the secretory pathway from the Golgi area to secretory granules in lacrimal glands, we notice (Fig. 7) that the patterns of radioactive protein courses in untreated and colchicine-treated cells are superimposable. Furthermore, we show that colchicine does not affect, at least obviously, the protein content composition, as deduced from protein molecular weight determination, evaluation of the relative proportions of each protein species in the glands (Fig. 4 and Table I), and the intracellular peroxidase activity. Together, these data consistently show that colchicine does not block the normal progress of secretory proteins up to secretory granules, as also pointed out in pancreas B-cells (37), but only delays the protein transport upstream to the Golgi area. It must be emphasized that this slowing down of the intracellular transport is a very short-term effect that can be demonstrated only when colchicine is added before the labeled-protein synthesis.

This delay cannot be assigned to structural alterations of the Golgi stacks described in various cells submitted to colchicine for 2 h or more (1, 14, 25, 30, 38, 39, 46, 47, 49, 53, 61, 66, 67), because under our conditions, the delay of protein transport is already detectable 25 min after the incubation onset, whereas the earliest Golgi saccule alterations are observed markedly later, from about 70 min onward. Further, the delay cannot be explained by some impairment of energy metabolism or of incorporation of amino acids (9) and sugar (data not shown) into proteins, because they are not altered by colchicine, as we have shown in lacrimal gland, and others have shown in different cells (fibroblasts: 12; chondrocytes: 33; hepatocytes: 3, 32, 43, 54, 63; parotid: 46; exocrine pancreas: 69). These results therefore suggest that colchicine may cause the delay of protein transport by acting on the cell compartments themselves, i.e., on their membrane or on their spatial distribution, mediated or not by the microtubular system (25).

INHIBITION OF PROTEIN RELEASE: LOCALIZATION OF THE COLCHICINE EFFECT: Secretory protein release has been reported to be inhibited or not by colchicine according to the experimental conditions (see 1, 26, 37, 64, 69). In the present work, we have shown that colchicine can cause a strong inhibition of either noninduced or carbachol-induced protein release[2], and that this effect is due neither to an alteration of cholinergic receptor activity (Figs. 2, 3 *a*, and reference 9) nor to a suppression of exocytosis, because (Fig. 2 *a*) colchicine does not decrease the induced peroxidase release during the first 30 min of stimulation.

This inhibition of secretory protein release was often thought to be produced by an alteration of the migratory pathway of formed secretory granules toward the cell apex, as shown in B-cells by Malaisse and Orci (36). We tried to test this idea, on lacrimal exorbital glands, by introducing colchicine at various times with respect to the pulse labeling. Fig. 5 indicates that a strong inhibition (75%) of protein release only occurs when colchicine is added 15–30 min before the pulse labeling. In contrast, inhibition is decreased to 55% when colchicine is added during or shortly after the pulse, then progressively diminishes when it is added later to reach ~5% after 50 min. In the colchicine-untreated cells, for the same times (compare Fig. 5 and Fig. 7), proteins are only reaching the Golgi area (30 min) or secretory granules (40 min). It thus appears that colchicine inhibits protein release by acting on the first steps of the secretory process when proteins are reaching the Golgi area or secretory granules, and not by acting on the last steps of the secretory process, as also seen in somatotrope cells (26). We must therefore suggest that, during the first steps of the secretory process, colchicine causes the secretory granules some incapacity to discharge their content.

[2] In lacrimal glands, the radiolabeled proteins are released in roughly constant proportions under carbachol stimulation (Fig. 5, Table I), as has been reported for the exocrine pancreas (65). A parallel release of several enzymes from pancreas or parotid glands under stimulated conditions has also been observed and discussed (28, 59). We show here that colchicine inhibits the release of each individual radiolabeled protein by the same percentage under either unstimulated or carbachol-stimulated conditions. Therefore, these results can be considered as an indication that the secreted labeled proteins come from the same intracellular pool, and are in agreement with the exocytosis model proposed by Palade (45) for secretion.

Fate of Nondischarged Secretory Granules

Biochemical investigations do not allow the detection of any significant modification in either β-*N*-acetylglucosaminidase or in cathepsin D activities (compare with 49 and 50), and products of ^{14}C-protein degradation are not discernible by polyacrylamide gel analysis. However, morphometric studies on colchicine-treated lacrimal glands show (Fig. 11) an increase of the relative surface of Golgi area-associated secondary lysosomes, as also reported qualitatively for many other cells: HeLa cells (56), pituitary gland (1), hepatocytes (53), mammary gland (41), and enterocytes (5). As these lysosomes, located in the Golgi area, are similar to secretory granules and never contain any recognizable cell organelles, we find that they reveal a selective destruction of secretory granules, a crinophagy process, as also found by Le Marchand et al. (32), and Reaven and Reaven (53) in hepatocytes.

In lacrimal glands, the significant rise of secondary lysosomes starts as soon as 50 min after incubation with colchicine has begun and strikingly increases thereafter (7). Radioactive labeling appears progressively in these lysosomes during the first 100 min of colchicine presence, but all lysosomes are labeled beyond 120 min. This suggests that crinophagy, quickly evoked by colchicine, first attacks nonlabeled secretory granules whose proteins have been synthesized before the pulse labeling.

An increase of lysosomes may be caused either by a secretory granule accumulation, as has already been suggested by many authors (1, 16, 22, 32, 46) or by a colchicine-induced anomaly of secretory granules. The results of Nickerson et al. (41) are in concordance with the first hypothesis. These authors have reported that, in mammary gland, colchicine induces a large accumulation of lysosomes only during in vitro incubation, but not in vivo. We suggest that in vivo the normal stimulation of discharge, even reduced by colchicine, is sufficient to suppress a strong accumulation of secretory granules and therefore crinophagy.

In conclusion, it appears that in vitro a low concentration (10 μM) of colchicine, which disrupts the labile microtubule system in lacrimal gland, does not suppress the secretory process but quickly affects it in two distinct ways. First, colchicine induces a slowing down of the rER-Golgi area secretory protein transport. Second, colchicine inhibits the discharge of secretory granules formed in its presence, but not of already formed granules. So colchicine acts mainly on the steps preceding granule formation, but clearly not on the steps following. On the other hand, these data suggest that in lacrimal exorbital gland the labile microtubule system could be implicated in the early steps of the secretory process before secretory granule formation without being indispensable for this process.

We wish to express our thanks to M.-C. Lainé-Delaunay and M. Louette for their skillful technical assistance.

This work was supported by the Centre National de la Recherche Scientifique (L.A. 0272), the Délégation Générale à la Recherche Scientifique et Technique and the Fondation pour la Recherche Médicale Française.

Correspondence and reprint requests should be addressed to Dr. Bernard Rossignol, Institut de Biochimie, Bt 432, Université de Paris Sud, 91405 ORSAY CEDEX, France.

Received for publication 6 January 1982, and in revised form 13 May 1982.

REFERENCES

1. Antalky, T., G. Pelletier, F. Zeytinoglu, and F. Labrie. 1979. Effects of colchicine on the morphology and prolactin secretion of rat anterior pituitary cells in monolayer culture. *Am. J. Anat.* 156:353–372.
2. Ashley, C. A., and T. Peters. 1967. Electron microscopic radioautographic detection of sites of protein synthesis and migration in liver. *J. Cell Biol.* 43:237–249.
3. Banerjee, D., C. P. Manning, and C. M. Redman. 1976. The in vivo effect of colchicine on the addition of galactose and sialic acid to rat hepatic serum glycoproteins. *J. Biol. Chem.* 251:3887–3892.
4. Barret, A. J., and M. F. Heath. 1977. *In* Lysosomes. A Laboratory Handbook. J. T. Dingle, editor. North-Holland Publishing Co. Amsterdam. 19–145.
5. Blok, J., L. A. Ginsel, A. A. Mulder-Stapel, J. J. M. Onderwater, and W. T. Daems. 1981. The effect of colchicine on the intracellular transport of ^{3}H-fucose-labelled glycoproteins in the absorptive cells of cultured human small-intestinal tissue. An autoradiographical and biochemical study. *Cell Tissue Res.* 215:1–12.
6. Boyenval, J., and J. Fischer. 1976. Dipping technique. *J. Microsc. Biol. Cell.* 27:115–119.
7. Busson-Mabillot, S., L. Ovtracht, A.-M. Chambaut-Guérin, P. Muller, G. Herman, and B. Rossignol. 1980. Protein secretion in rat exorbital lacrimal gland: in vitro effects of colchicine. *Eur. J. Cell Biol.* 22:184.
8. Castle, J. D., J. D. Jamieson, and G. E. Palade. 1972. Radioautographic analysis of the secretory process in the parotid acinar cell of the rabbit. *J. Cell Biol.* 53:290–311.
9. Chambaut-Guérin, A.-M., P. Muller, and B. Rossignol. 1978. Microtubules and protein secretion in rat lacrimal glands. Relationship between colchicine binding and its inhibitory effect on the intracellular transport of proteins. *J. Biol. Chem.* 253:3870–3876.
10. Chambaut-Guérin, A.-M., P. Muller, and B. Rossignol. 1979. Microtubules and protein secretion in rat lacrimal glands. Inhibitory effect of the tubulin-colchicine complex isolated from lacrimal glands upon brain tubulin polymerization. Identification of the complex by gel electrophoresis. *J. Biol. Chem.* 254:10734–10739.
11. Chu, L. L. H., R. R. MacGregor, and D. V. Cohn. 1977. Energy-dependent intracellular translocation of proparathormone. *J. Cell Biol.* 72:1–10.
12. Diegelmann, R. F., and B. Peterkofsky. 1972. Inhibition of collagen secretion from bone and cultured fibroblasts by microtubular disruptive drugs. *Proc. Natl. Acad. Sci. U. S. A.* 69:892–896.
13. Droz, B. 1976. Kinetic analysis of macromolecular movements in cells by means of radioautography. *J. Microsc. Biol. Cell.* 27:191–200.
14. Ehrlich, H. P., R. Ross, and P. Bornstein. 1974. Effects of antimicrotubular agents on the secretion of collagen. A biochemical and morphological study. *J. Cell Biol.* 62:390–405.
15. Essner, E. 1971. Localization of endogenous peroxidase in rat exorbital lacrimal gland. *J. Histochem. Cytochem.* 19:216–225.
16. Farquhar, M. G. 1971. Processing of secretory products by cells of the anterior pituitary gland. *In* Subcellular Organization and Function in Endocrine Tissues. *Mem. Soc. Endocrinol.* 19:79–124.
17. Feldmann, G., M. Maurice, C. Sapin, and J.-P. Benhamou. 1975. Inhibition by colchicine of fibrinogen translocation in hepatocytes. *J. Cell Biol* 67:237–243.
18. Flickinger, C. J. 1974. Synthesis, intracellular transport, and release of secretory protein in the seminal vesicle of the rat, as studied by electron microscope radioautography. *Anat. Rec.* 180:407–426.
19. Flickinger, C. J. 1974. Protein secretion in the rat ventral prostate and the relation of Golgi vesicles, cisternae and vacuoles, as studied by electron microscope radioautography. *Anat. Rec.* 180:427–448.
20. Glaumann, H., and J. L. E. Ericsson. 1970. Evidence for the participation of the Golgi apparatus in the intracellular transport of nascent albumin in the liver cell. *J. Cell Biol.* 47:555–567.
21. Habener, J. F., M. Amherdt, M. Ravazzola, and L. Orci. 1979. Parathyroid hormone biosynthesis. Correlation of conversion of biosynthetic precursors with intracellular protein migration as determined by electron microscope autoradiography. *J. Cell Biol.* 80:715–731.
22. Habener, J. F., B. Kemper, and J. T. Potts. 1975. Calcium-dependent intracellular degradation of parathyroid hormone: a possible mechanism for the regulation of hormone stores. *Endocrinology.* 97:431–441.
23. Herman, G., S. Busson, L. Ovtracht, C. Maurs, and B. Rossignol. 1978. Regulation of protein discharge in two exocrine glands: rat parotid and exorbital lacrimal glands. Analogies between cholinergic (muscarinic) and α-adrenergic stimulation and importance of extracellular calcium. *Biol. Cell.* 31:255–264.
24. Herzog, V., H. Sies, and F. Miller. 1976. Exocytosis in secretory cells of rat lacrimal gland. Peroxidase release from lobules and isolated cells upon cholinergic stimulation. *J. Cell Biol.* 70:692–706.
25. Hoffstein, S., I. Goldstein, and G. Weissmann. 1977. Role of microtubule assembly in lysosomal enzyme secretion from human polymorphonuclear leukocytes: a reevaluation. *J. Cell Biol.* 73:242–256.
26. Howell, S. L., and M. Tyhurst. 1978. Role of microtubules in the intracellular transport of growth hormone. *Cell Tissue Res.* 190:163–171.
27. 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.
28. Kanagasuntheram, P., and S. C. Lim. 1981. Parallel secretion of secretory proteins and calcium by the rat parotid gland. *J. Physiol. (Lond.)* 312:445–454.
29. Kemper, B., J. F. Habener, A. Rich, and J. P. Potts. 1975. Microtubules and the intracellular conversion of proparathyroid hormone to parathyroid hormone. *Endocrinology.* 96:903–912.
30. Knudson, C. M., B. H. Stemberger, and S. Patton. 1978. Effects of colchicine on ultrastructure of the lactating mammary gland: membrane involvement and stress on the Golgi apparatus. *Cell Tissue Res.* 195:169–181.
31. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.
32. Le Marchand, Y., A. Singh, F. Assimacopoulos-Jeannet, L. Orci, C. Rouiller, and B. Jeanrenaud. 1973. A role for the microtubular system in the release of very low density lipoproteins by perfused mouse livers. *J. Biol. Chem.* 248:6862–6870.
33. Lohmander, S., S. Moskalewski, K. Madsen, J. Thyberg, and U. Friberg. 1976. Influence of colchicine on the synthesis and secretion of proteoglycans and collagen by fetal guinea pig chondrocytes. *Exp. Cell Res.* 99:333–345.
34. 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.
35. Malaisse, W. J., F. Malaisse-Lagae, E. Van Obberghen, G. Somers, G. Devis, M. Ravazzola, and L. Orci. 1975. Role of microtubules in the phasic pattern of insulin release. *Ann. N. Y. Acad. Sci.* 253:630–652.
36. Malaisse, W. J., and L. Orci. 1979. The role of the cytoskeleton in pancreatic B-cell function. *Methods Achiev. Exp. Pathol.* 9:112–136.
37. Malaisse-Lagae, F., M. Amherdt, M. Ravazzola, A. Sener, J. C. Hutton, L. Orci, and W. J. Malaisse. 1979. Role of microtubules in the synthesis, conversion, and release of (pro)insulin. A biochemical and radioautographic study in rat islets. *J. Clin. Invest.* 63:1284–1296.

38. Moskalewski, S., J. Thyberg, and U. Friberg. 1976. In vitro influence of colchicine on the Golgi complex in A- and B-cells of guinea-pig pancreatic islets. *J. Ultrastruct. Res.* 54:304–317.
39. Moskalewski, S., J. Thyberg, S. Lohmander, and U. Friberg. 1975. Influence of colchicine and vinblastine on the Golgi complex and matrix deposition in chondrocyte aggregates. An ultrastructural study. *Exp. Cell Res.* 95:440–454.
40. Nadler, N. J. 1979. Quantitation and resolution in electron microscope radioautography. *J. Histochem. Cytochem.* 27:1531–1533.
41. Nickerson, S. C., J. J. Smith, and T. W. Keenan. 1980. Role of microtubules in milk secretion—action of colchicine on microtubules and exocytosis of secretory vesicles in rat mammary epithelial cells. *Cell Tissue Res.* 207:361–376.
42. Ollivier-Bousquet, M., and R. Denamur. 1973. Inhibition par la colchicine de la sécrétion des protéines du lait. *C. R. H. Acad. Sci. (Paris) Ser. D.* 276:2183–2186.
43. Orci, L., Y. Le Marchand, A. Singh, F. Assimacopoulos-Jeannet, C. Rouiller, and B. Jeanrenaud. 1973. Role of microtubules in lipoprotein secretion by the liver. *Nature (Lond.).* 244:30–32.
44. Orci, L., A. A. Like, M. Amherdt, B. Blondel, Y. Kanazawa, E. B. Marliss, A. E. Lambert, C. B. Wollheim, and A. E. Renold. 1973. Monolayer culture of neonatal rat pancreas: an ultrastructural and biochemical study of functioning endocrine cells. *J. Ultrastruct. Res.* 43:270–297.
45. Palade, G. E. 1975. Intracellular aspects of the process of protein secretion. *Science (Wash. D. C.).* 189:347–358.
46. Patzelt, C., D. Brown, and B. Jeanrenaud. 1977. Inhibitory effect of colchicine on amylase secretion by rat parotid glands. Possible localization in the Golgi area. *J. Cell Biol.* 73:578–593.
47. Pavelka, M., and A. Ellinger. 1981. Effect of colchicine on the Golgi apparatus and on GERL of rat jejunal absorptive cells. Ultrastructural localization of thiamine pyrophosphatase and acid phosphatase activity. *Eur. J. Cell Biol.* 24:53–61.
48. Pelletier, G., and M. B. Bornstein. 1972. Effect of colchicine on rat anterior pituitary gland in tissue culture. *Exp. Cell Res.* 70:221–223.
49. Piasek, A., and J. Thyberg. 1979. Effects of colchicine on endocytosis and cellular inactivation of horseradish peroxidase in cultured chondrocytes. *J. Cell Biol.* 81:426–437.
50. Piasek, A., and J. Thyberg. 1980. Effects of colchicine on endocytosis of horseradish peroxidase by rat peritoneal macrophages. *J. Cell Sci.* 45:59–71.
51. Putney, J. W., C. M. Van de Walle, and B. A. Leslie. 1978. Stimulus-secretion coupling in the rat lacrimal gland. *Am. J. Physiol.* 235:c188–198.
52. Reaven, E. P., and G. M. Reaven. 1978. Dissociation between rate of hepatic lipoprotein secretion and hepatocyte microtubule content. *J. Cell Biol.* 77:735–742.
53. Reaven, E. P., and G. M. Reaven. 1980. Evidence that microtubules play a permissive role in hepatocyte very low density lipoprotein secretion. *J. Cell Biol.* 84:28–39.
54. Redman, C. M., D. Banerjee, K. Howell, and G. E. Palade. 1975. Colchicine inhibition of plasma protein release from rat hepatocytes. *J. Cell Biol.* 66:42–59.
55. Redman, C. M., D. Banerjee, C. Manning, C. Y. Huang, and K. Green. 1978. In vivo effect of colchicine on hepatic protein synthesis and on the conversion of proalbumin to serum albumin. *J. Cell Biol.* 77:400–416.
56. Robbins, E., and N. K. Gonatas. 1964. Histochemical and ultrastructural studies on HeLa cell cultures exposed to spindle inhibitors with special reference to the interphase cell. *J. Histochem. Cytochem.* 12:704–711.
57. Rohr, H.-P., J. Schmalbeck, and A. Feldhege. 1967. Elektronenmikroskopisch-autoradiographische Untersuchungen über die Eiweiss-Synthese in der Brunnerschen Drüse der Maus. *Z. Zellforsch. Mikrosk. Anat.* 80:183–204.
58. Rohr, H.-P., U. Seitter, and J. Schmalbeck. 1968. Voraussetzengen und derzeitige Grenzen der quantitativen elektronenmikroskopischen autoradiographie bei kinetikstudien an Drusenzellen. Elektronenmikroskopisch-autoradiographische Untersuchungen mit ^{3}H-Leucin an der Milchdrüse. *Z. Zellforsch. Mikrosk. Anat.* 85:376–397.
59. Scheele, G. A. 1980. Biosynthesis, segregation, and secretion of exportable proteins by the exocrine pancreas. *Am. J. Physiol.* 238:G467–G477.
60. Sellinger, O. Z., H. Beaufay, P. Jacques, A. Doyen, and C. de Duve. 1960. Tissue fractionation studies. 15. Intracellular distribution and properties of β-*N*-acetyl-glucosaminidase and β-galactosidase in rat liver. *Biochem. J.* 74:450–456.
61. Seybold, J., W. Bieger, and H. F. Kern. 1975. Studies on intracellular transport of secretory proteins in the rat exocrine pancreas. II. Inhibition by antimicrotubular agents. *Virchows Arch. A Pathol. Anat. Histol.* 368:309–327.
62. Soifer, D., editor. 1975. The biology of cytoplasmic microtubules. *Ann. N. Y. Acad. Sci.* 253: Pts. X and XII.
63. Stein, O., L. Sanger, and Y. Stein. 1974. Colchicine-induced inhibition of lipoprotein and protein secretion into the serum and lack of interference with secretion of biliary phospholipids and cholesterol by rat liver in vivo. *J. Cell Biol.* 62:90–103.
64. Stein, O., and Y. Stein. 1973. Colchicine-induced inhibition of very low density lipoprotein release by rat liver in vivo. *Biochim. Biophys. Acta.* 306:142–147.
65. Tartakoff, A. M., J. D. Jamieson, G. A. Scheele, and G. E. Palade. 1975. Studies on the pancreas of the guinea pig. Parallel processing and discharge of exocrine proteins. *J. Biol. Chem.* 250:2671–2677.
66. Thyberg, J., S. Nilsson, S. Moskalewski, and A. Hinek. 1977. Effects of colchicine on the Golgi complex and lysosomal system of chondrocytes in monolayer culture. An electron microscopic study. *Cytobiologie.* 15:175–191.
67. Thyberg, J., A. Piasek, and S. Moskalewski. 1980. Effects of colchicine on the Golgi complex and GERL of cultured rat peritoneal macrophages and epiphyseal chondrocytes. *J. Cell Sci.* 45:42–58.
68. Weinstock, M., and C. P. Leblond. 1974. Synthesis, migration, and release of precursor collagen by odontoblasts as visualized by radioautography after (^{3}H)proline administration. *J. Cell Biol.* 60:92–127.
69. Williams, J. A., and M. Lee. 1976. Microtubules and pancreatic amylase release by mouse pancreas in vitro. *J. Cell Biol.* 71:795–806.