GLYCOPROTEIN TRANSPORT IN THE SURFACE MUCOUS CELLS OF THE RAT STOMACH

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ABSTRACT

The intracellular transport of glycoproteins pulse-labeled in vitro with tritiated leucine and galactose in the surface mucous lining cells (SMC) of the fundus of the rat stomach was studied by electron microscope autoradiography. The SMC survive for several hours in pieces of the fundus incubated in a bicarbonate-buffered medium. The SMC have a normal ultrastructure for at least 4 h of incubation. Kinetic activity is normal for at least 5 h, as demonstrated by the normal nuclear incorporation of tritiated thymidine. The SMC incorporate labeled leucine and galactose at normal rates up to 4 h and 6 h, respectively. In contrast to the SMC, the cells of the gastric glands show signs of degeneration within 1 h after the start of incubation.

In the SMC the secretory protein forms a smaller part of the total protein synthesized than in other secretory cells studied. The intracellular transport of the leucine-labeled moiety of the glycoproteins follows the normal pathway. The RER loses 35% of its transportable labeled protein within 30 min. The Golgi complex is maximally labeled at 40 min and the mucous granules after 120 min. Galactose is attached to the glycoproteins mainly in the Golgi complex. Glycoproteins are not secreted within 2 h after synthesis of their protein moiety.

The intracellular transport of secretory protein and the protein and carbohydrate moieties of secretory glycoproteins has been described in a number of cell types. However, the pathway of glycoprotein synthesis and transport in the surface mucous cells (SMC) of the stomach has not been studied. The SMC form the mucus-secreting epithelial lining of the stomach and follow this lining into the gastric pits. For the study of intracellular transport in the SMC, biochemical methods are not desirable because cellular material obtained by scraping or by vibration of the mucosal wall does not only contain the SMC but also mucous neck cells and pepsinogen cells. Furthermore, mucous glycoproteins in the homogenate hinder the preparation of subcellular fractions. We therefore decided to study glycoprotein transport in rat SMC by electron microscope autoradiography. Preliminary studies showed that in these SMC the rate of incorporation of radioactive amino acid or sugar is too low to be detected in electron microscope autoradiographs unless very high doses of the precursors are administered. However, the SMC appeared to be capable of incorporating these precursors in vitro. Hence, after preliminary experiments to determine the survival of the SMC under the chosen conditions, glycoprotein transport was studied in incubated pieces of the fundus of the rat stomach.

PRELIMINARY EXPERIMENTS

Procedures

ANIMALS: Male Wistar albino rats (Centraal Proefdierenbedrijf TNO, Zeist, Nederland), weighing

about 300 g, were starved for 24 h and killed by decapitation. The stomach was freed, opened along the lesser curvature from the duodenum to the esophagus, and pinned onto a wax plate. Eight 2 × 3-mm pieces of tissue were excised from the fundic part of the stomach along a line parallel to the border between the blind sac and the fundus. These pieces were put into the preincubation vial within 4 min after the death of the animal.

INCUBATION PROCEDURE: The pieces of tissue floated in 2 ml of a bicarbonate-buffered medium (pH 7.6) (15) which included: amino acids at blood plasma concentration (19), adenosine, cytidine, guanosine, and uridine at 0.1 mM, and 3-hydroxy-butyrate at 5.7 mM (1). The concentration of glucose was 5.4 mM. After preincubation the pieces of tissue were transferred into another vial containing 2 ml of the same medium with the radioactive precursor. At the end of the incubation, the specific radioactivity of the precursor was diluted by adding nonradioactive precursor. After two washings in 4 ml of medium, the pieces were postincubated for various times, if needed. All incubations were performed at 38°C. The vials were gassed with a warmed, humidified mixture of 95% O_2 and 5% CO_2 and gently shaken.

TISSUE PROCESSING FOR MICROSCOPY: At the end of the (post)incubation, the pieces were fixed for 24 h in 4% (wt/wt) formaldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C. They were rinsed for 24 h in the same buffer with 6.8% (wt/wt) sucrose at 4°C, and then dehydrated and embedded in paraffin.

For electron microscopy, tissue fragments were immersed in a drop of fixation fluid, sliced with a tissue sectioner, fixed in either 1% OsO_4 in cacodylate buffer (pH 7.4) or a mixture of 2% formaldehyde and 2% glutaraldehyde in phosphate buffer (pH 7.9; 970 mosM), and postfixed in 1% OsO_4. After dehydration, the slices were transferred to flat embedding trays filled with Epon 812. The slices were oriented for sectioning perpendicular to the mucosal surface. Grayish sections were stained with lead citrate and studied in a Siemens Elmiskop I electron microscope.

Tests on the Experimental Model

To investigate the viability of the SMC in the in vitro conditions, we performed a number of experiments. The parameters of survival used were: the cellular ultrastructure, the number of cells incorporating [^{3}H]thymidine, the rate of incorporation of L-[^{3}H]leucine and D-[^{3}H]galactose into the SMC, and the loss of radioactive protein into the medium during postincubation.[1]

The ultrastructure of the SMC shows little changes during the first 4 h of incubation (see below).

INCORPORATION OF [^{3}H]THYMIDINE: Pieces of fundus were incubated in medium containing [*methyl*-^{3}H]thymidine (The Radiochemical Centre, Amersham, England); specific radioactivity 5 Ci/mmole) for 30 min after a preincubation period of 10 min or 4.5 h. At the end of the radioactive incubation, the pieces were fixed and embedded in paraffin. Sections 5 μm thick were cut perpendicular to the mucosal surface and parallel to the fundic glands. The sections were covered with Kodak AR 10 stripping film, then dried and stored in a refrigerator for 14 days. The film was developed in D 19 b at 18°C, and fixed in a sodium thiosulfate (10% wt/wt) and potassium metabisulphite (1% wt/wt) solution in distilled water. The preparations were stained with methyl green-pyronin for 6 min and differentiated in 70% ethanol.

The number of cells that incorporated radioactive thymidine above background, i.e., more than four silver grains per nucleus, was counted per longitudinally sectioned pit and gland. 30 pits with glands per section were scored. The mean number of labeled nuclei per pit and gland is called the labeling index.

To compare the labeling index between glands in vivo and in vitro, we injected tritiated thymidine into the tail vein of three rats. The rats were killed 1 h after the injection. The labeling index, determined in autoradiographs of the same fundic area from which the pieces for incubation experiments were taken, varied from 1.7 to 3.0 (Table I). After incubation with [^{3}H]thymidine, the labeling index varied between 0.6 and 2.2. When the pieces of fundus were incubated for 4.5 h before the labeled precursor was added, the mean number of cells in S phase per pit and gland was 2.6–3.1. Fig. 1 shows the distribution of the number of S cells per pit and gland section in vivo and in vitro. The higher value after long incubation might result from shrinkage of the tubules since a narrow lumen allows more cells to be present in a longitudinal section.

Incubation for about 5 h apparently does not decrease the kinetic capacity of the epithelial cells at the transition between pits and glands.

INCORPORATION OF [^{3}H]LEUCINE: Pieces of fundus were incubated in medium containing L-[4.5-^{3}H]leucine (The Radiochemical Centre; specific radioactivity 19–22 Ci/mmole) for 10 min after preincubation periods varying between 10 min and 6 h. At the end of the radioactive incubation the pieces were processed for light microscope autoradiography (exposure time: 10 days). In one section of each piece of fundus the number of silver grains over a part of the epithelial lining (pits included) was counted and divided by the number of nuclei counted in the same part. This quotient gives the

[1] The continuous production of [^{14}C]O_2 by the pieces of fundus in a medium containing 10 μCi of D-[U-^{14}C]glucose (The Radiochemical Centre, specific radioactivity 335 mCi/mmole) up to at least 6 h in the absence of bacterial contamination cannot be used as an index of cell survival since the mitochondria continue their oxidative phosphorylation even after release from disintegrating cells. In fact, parietal cells with their large numbers of mitochondria are frequently seen disintegrating (see Fig. 6).

TABLE I

[³H]Thymidine Labeling Index of Fundic Epithelium In Vivo and In Vitro*

Rat no.	In vivo‡	In vitro§ preincubation time	
		10 min	4.5 h
1		2.2 (1)	2.6 (3)
2		1.2 (2)	3.1 (2)
3		0.6 (5)‖	
4	1.7 (3)		
5	3.0 (3)		
6	2.2 (32)¶		

* Each value represents the labeling index (mean number of labeled nuclei per pit and gland) of one rat, the number of sections studied being mentioned within brackets. 30 pits were screened in each section.

‡ 1 h after [³H]thymidine injection (1 μCi/g body weight, i.v.).

§ Pieces of fundus were preincubated in 2 ml of Krebs (1932) bicarbonate-buffered medium with supplements. After 10 min or 4.5 h, 50 μCi [³H]thymidine was added for 30 min. Thereafter, the pieces were washed with medium with a 200-fold concentration of nonradioactive thymidine, and fixed for light microscopy.

‖ The variation coefficient of these five sections amounted to 18.5%.

¶ The variation coefficient of these 32 sections amounted to 18.2%.

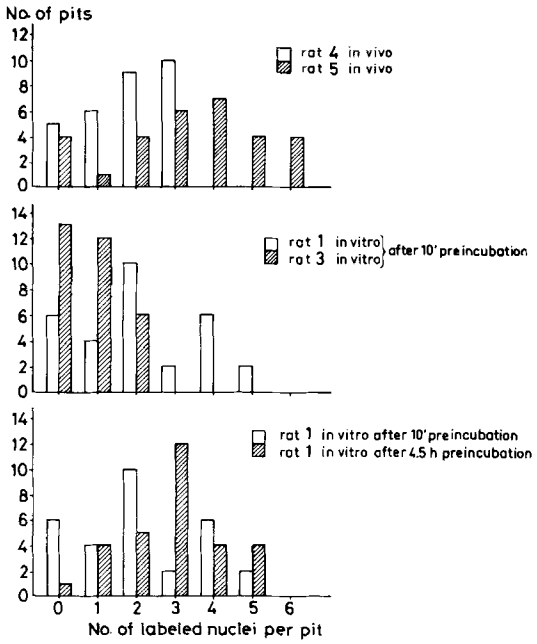


FIGURE 1 Distribution of the number of labeled nuclei per pit after administration of [³H]thymidine in vivo or in vitro. The mean values per rat and the number of sections studied are given in Table I. For further details, see notes to Table I (the rats bear the same numbers).

number of silver grains per mean cellular area. The mean of these quotients for several fields from one section is used as parameter of the rate of incorporation of leucine in that piece of fundus.

In six of the seven experiments, the SMC showed no significant loss of incorporating capacity within 4 h after the onset of incubation (Fig. 2; the exception is exp C). Generally, incubation for 4 h does not decrease the protein-synthesizing capacity of the SMC.

The number of silver grains per mean cellular area varied greatly among the experiments (Fig. 2), and, to a lesser degree, among sections from a single piece of fundus and even between areas within one section. The variation coefficient of five sections from five pieces from one stomach was 31–56%. The epithelial cells near the cut surfaces of the tissue pieces incorporate far more [³H]leucine than the lining cells farther from the cut surfaces. Therefore, countings were made in areas that were more than 50 cells aways from the cut surface.

The incorporation might vary because of a more or less thick layer of undissolved mucus that coats the luminal surfaces of the epithelial cells. This mucus layer might be a barrier to the diffusion of substances between medium and SMC. The high rate of incorporation at the cut surfaces where the mucus layer can be circumvented supports this explanation.

Only cells lying above the transitional zone between pits and glands incorporate [³H]leucine; cells below this

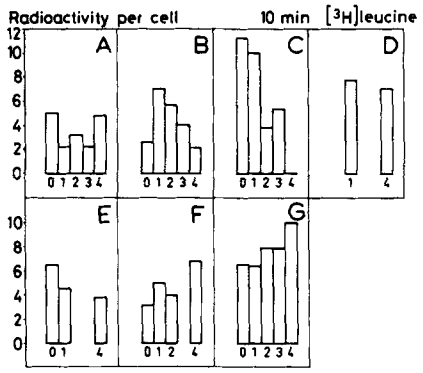


FIGURE 2 Influence of incubation time on the incorporation of [³H]leucine in vitro. Pieces of fundus were preincubated in 2 ml of Krebs (15) bicarbonate-buffered medium with supplements. After 10 min, 1, 2, 3, or 4 h, 100 μCi [³H]leucine was added for 10 min (these preincubation periods are mentioned below each column). Thereafter, 2 ml of medium with 8.3 mg of unlabeled leucine were added, and the pieces were fixed for light microscopy. Each column represents the incorporation value (no. of silver grains per mean cellular area) in a piece of fundus. Each figure (A–G) represents the results from one rat.

zone, including the pepsinogen cells, do not. Only close to the cut surfaces the deeper cells, especially the pepsinogen cells, show incorporation.

INCORPORATION OF [^{3}H]GALACTOSE: Pieces of fundus were incubated in 2 ml of medium containing 50 μCi of D-[1-^{3}H]galactose (The Radiochemical Centre; specific radioactivity 18.4 Ci/mmole) for 10 min after a preincubation period of 10 min or 6 h. At the end of the radioactive incubation the specific radioactivity was diluted 200-fold by adding 2 ml of medium with 1 mg of unlabeled galactose to the medium.

The pieces of tissue processed for autoradiography were exposed for 10 days. The number of silver grains per mean cellular area in eight pieces from two stomachs was 4.9 (SEM = 0.6) after 10 min of preincubation, and 4.3 (SEM = 0.2) in four pieces from two stomachs after 6 h of preincubation. This indicates that incubation up to 6 h does not impair the synthetic capacity of the SMC.

[^{3}H]Galactose, too, is not incorporated by cells below the level of the gastric pits. Its incorporation varied less than that of [^{3}H]leucine: the variation coefficient of five sections from five pieces from one stomach was between 3% and 25%.

LOSS OF RADIOACTIVE (GLYCO)PROTEIN FROM THE EPITHELIAL CELLS: If cells that have incorporated radioactive amino acid or sugar into (glyco)protein should disintegrate later in the incubation period, then radioactive protein should leak into the medium. Since the SMC are the only cells that incorporate radioactive amino acids and galactose, a sharp increase in radioactive (glyco)protein in the medium should indicate that many SMC have died. Conversely, a gradual increase should occur during normal cellular turnover.

The amount of protein radioactivity in the medium was measured at intervals between 20 min and 6 h after the introduction of radioactive precursor. Pieces of fundus were incubated for 3 min in medium containing [^{3}H]leucine or [^{3}H]galactose, washed and postincubated in nonradioactive medium. At various times, aliquots were taken from the medium for measurement of the trichloroacetic acid (TCA)-insoluble and the TCA-soluble radioactivity. After addition of albumin, the protein was precipitated by TCA at a final concentration of 10%. The amount of radioactivity in both the TCA-soluble and TCA-insoluble fractions was measured by dioxane-based scintillation at an efficiency of 17%. The results of two of the six experiments with [^{3}H]leucine are shown in Fig. 3. Little radioactivity was transferred from cells to the medium. The transfer rate diminished during the experiment and showed no sign of an abrupt increase.

To determine whether massive cell death leads to a concurrent increase in the amount of radioactive protein in the medium, we replaced the gassing mixture of 95% O_2 and 5% CO_2, after 2-h incubation, by pure nitrogen for an additional 4 h. Indeed, more than 1 h after N_2 administration, large amounts of radioactive protein stream into the medium (Fig. 4).

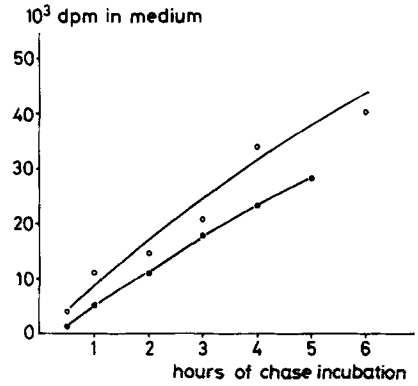


FIGURE 3 Amounts of radioactivity in protein in the medium during chase incubation after pulse labeling with [^{3}H]leucine. Pieces of fundus (total weight about 100 mg) were incubated for 3 min in 2 ml of medium containing 100 μCi [^{3}H]leucine, washed, and postincubated in nonradioactive medium, from which aliquots were taken at various intervals (abscissa) for measuring the amount of TCA-insoluble radioactivity. Exp 1 (●——●); exp 2 (○——○).

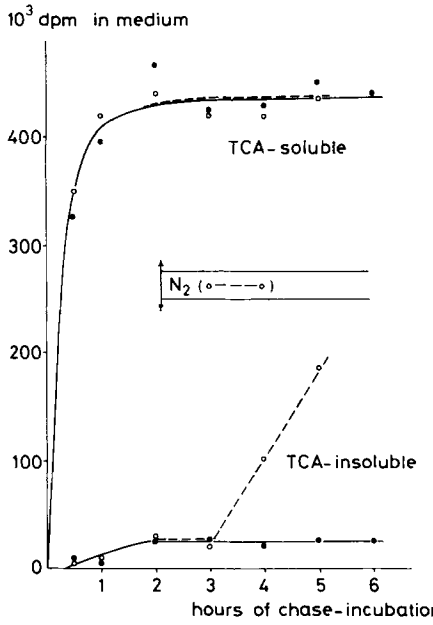


FIGURE 4 Effect of cell death on the amount of radioactivity in the medium during chase incubation after a 3-min pulse labeling with [^{3}H]leucine. After 2 h of chase incubation, cell death was induced by replacing the gassing mixture of oxygen and carbon dioxide by pure nitrogen (○-----○). In a parallel experiment (same amount and weight of pieces of fundus from the same rat), incubation was continued without nitrogen, to serve as a control (○——○). Lower graph: TCA-insoluble radioactivity; upper graph: TCA-soluble radioactivity. For further details, see legend to Fig. 3.

The loss of [^{3}H]galactose glycoprotein into the medium appeared to be small: nil after 4 h of postincubation in three experiments, and 14,000 and 20,000 dpm per 100 mg fundic tissue after 6 h in two experiments in which 50 μCi of [^{3}H]galactose was offered to the tissue in 2 ml of medium for 10 min. Apparently, the glycoprotein molecules start to be secreted late (between 4 and 6 h), or they are secreted earlier but form part of the "insoluble" mucous film adhering to the fundic lining before dissolving into the medium (see below).

From these experiments, we conclude that most of the SMC survived 6 h of incubation.

IDENTIFICATION OF [^{3}H]LEUCINE AND [^{3}H]GALACTOSE: Thin-layer chromatography followed by liquid scintillation of the scraped leucine spot indicated that the radioactive leucine added to the medium was not metabolized into other radioactive compounds during the experiments. At the end of a 10-min [^{3}H]leucine incorporation period, radioactivity of the TCA-soluble material from the pieces of stomach was present for more than 90% in the leucine area of the chromatographs as was the radioactivity of the TCA-insoluble material after hydrolyzation in 6 N HCl at 110°C.

At the end of a 10-min [^{3}H]galactose incorporation period, radioactivity of the TCA-insoluble material from the pieces of stomach was present only in D-galactose as appeared from a gas chromatographic analysis of the hydrolysate of this material.

MAIN EXPERIMENTS

Methods

INCUBATION: Five pieces of fundus from one stomach were incubated in medium containing a high concentration of [^{3}H]leucine (500 μCi/ml) or D-[1-^{3}H]galactose (250 μCi/ml). Incubation in radioactive medium for 3 or 5 min was followed by thorough washing in nonradioactive medium containing a higher concentration of nonradioactive leucine or galactose. The pieces were put into the postincubation vial and, at the times indicated, processed for electron microscopy and electron microscope autoradiography.

For comparison of the ultrastructural appearance of the incubated cells, another piece of fundus was fixed immediately after excision from the stomach.

The experiment was repeated four times with [^{3}H]leucine and three times with [^{3}H]galactose. Hence, the values of Tables II and III are the weighed means of countings in sections from four and three rats, respectively. The periods of labeling and chase incubation are shown in these tables.

ELECTRON MICROSCOPE AUTORADIOGRAPHY: For electron microscope autoradiography, gold sections of osmium tetroxide-fixed tissue were placed on collodion-covered slides, stained with lead citrate, and coated with a thin layer of carbon. A layer of Ilford L 4 photographic emulsion, one to two crystals thick of silver bromide, was laid on the section by dipping the slides into the emulsion with the aid of a dipping device (14, 37). After 1–3 mo exposure at 4°C, the autoradiographs were developed in Phenidone (Ilford Ltd., Ilford, Essex, England) for 1 min at 16°C (18) and fixed in 20% sodium thiosulphate. The collodion film with sections and emulsion was floated onto water and picked up on uncoated copper grids.

PHENIDONE DEVELOPMENT:[2] In monolayer autoradiographs of 100-nm thick sections of [^{3}H]-methacrylate, the number of silver deposits developed by Phenidone appeared to increase linearly with exposure time (radiation dose) up to 60 days, when a density of 14 grains/μm^2 was reached. When we used a tritiated line source, the half distance was about 160 nm. A silver bromide crystal gives rise to only one silver deposit within its perimeter. Background over nonradioactive control sections was negligible. In the autoradiographs of fundic cells, chains or clusters of silver deposits are found over areas of presumed high radioactive density (see Fig. 11), but not over areas where it is low (RER). This indicates that the formation of deposits during development does not propagate from a hit crystal to the nonhit adjacent crystals. The chains are simply the result of local high frequencies of developed crystals over areas of high radioactivity.

QUANTITATIVE ELECTRON MICROSCOPE AUTORADIOGRAPHY: Silver grains were counted in electron micrographs of randomly selected SMC. The number of grains per cellular compartment is expressed as a percentage of the total number of grains counted (relative amount of silver grains).

The RER category includes silver grains lying above RER cisternae, membranes, and attached ribosomes. The Golgi complex includes the cisternae and the area of vesicles and tubules of both the entry and the exit sides of the Golgi stacks. Condensing vacuoles and young secretory granules, not yet added to the compact mass of mature granules lying in the cell top, are taken together. Cytoplasmic matrix also includes the free ribosomes and the bundles of filaments.

The volume density of the various compartments, mentioned in Fig. 11, was measured for each of the postincubation times, by placing a transparent screen over the pictures of EM autoradiographs (× 24,000). The screen was marked by 1-cm-spaced lines. The intersections of these lines (test points) overlying the compartments were counted. The volume density of each type of cell structure is the quotient of the number of test points over the structure and the total number of test points over surface mucous cells. By dividing the relative amount of silver grains over a cellular compartment by its volume density, we calculated the relative concentration of radioactive (glyco)protein in that compartment.

ASPECIFIC BINDING OF RADIOACTIVE PRECURSORS BY THE FIXATIVE: Apart from being

[2] The detailed results of a study of the quantitative characteristics of Phenidone development will be published elsewhere.

TABLE II

Distribution of Autoradiographic Grains over Intracellular Structures after a Pulse-Labeling with [^{3}H]leucine

	Pulse + chase incubation times in minutes*					
	3 + 2	5 + 5	5 + 15	5 + 35	5 + 55	5 + 115
	% of autoradiographic grains					
RER	*32.2*	26.0	22.1	17.3	12.6	8.5
Golgi complex	11.4	17.4	23.0	*28.7*	25.1	10.5
Cisternae, tubules, vesicles	9.0	15.6	19.7	21.8	16.8	6.4
Condensing vacuoles‡	2.3	2.0	3.3	6.0	8.3	3.9
Mucous granules	4.1	3.6	4.2	7.0	16.3	*39.1*
Nuclei + remainder of cytoplasm	52.3	40.6	50.4	47.0	46.1	42.1
No. of grains counted	1,247	1,463	1,352	1,684	1,713	1,470

* Pieces of fundus were preincubated at 37°C in 2 ml of Krebs (1932) bicarbonate-buffered medium with supplements. After 10 min, 1,000 μCi L-[^{3}H]leucine was added for 3 or 5 min. The pieces were washed twice with medium with a 200-fold concentration of nonradioactive leucine, and postincubated in nonradioactive medium for various times.

‡ Including youngest mucous granules.

TABLE III

Distribution of Autoradiographic Grains over Intracellular Structures after a Pulse-Labeling with [^{3}H]Galactose

	Pulse + chase incubation times in minutes*				
	3 + 2	5 + 5	5 + 15	5 + 35	5 + 55
	% of autoradiographic grains				
RER	*13.4*	6.4	7.9	3.4	3.8
Golgi complex	61.6	*71.8*	65.2	46.4	25.9
Cisternae, tubules, vesicles	46.7	33.5	30.4	16.1	7.1
Condensing vacuoles‡	14.9	38.3	34.8	30.3	18.8
Mucous granules	2.1	2.3	7.9	23.4	*43.5*
Nuclei + remainder of cytoplasm	22.9	19.5	19.0	26.8	26.8
No. of grains counted	336	561	517	584	522

* Pieces of fundus were preincubated at 37°C in 2 ml of Krebs (1932) bicarbonate-buffered medium with supplements. After 10 min, 500 μCi D-[^{3}H]galactose was added for 3 or 5 min. The pieces were washed twice with a 200-fold concentration of nonradioactive galactose, and postincubated in nonradioactive medium for various times.

‡ Including youngest mucous granules.

incorporated into glycoproteins, leucine and galactose might be bound aspecifically to the tissue components. If this radioactivity should form a great proportion of the total radioactivity in the sections, the results of the quantitative autoradiography should be difficult to interpret. To measure the relative amount of the aspecific radioactivity, we incubated pieces of gastric fundus for 5 min in a medium containing 500 μCi [^{3}H]leucine per ml, as used in the main experiments. One piece was incubated at 37°C, a second at 37°C in the presence of 10^{-3} M puromycin-HCl, and a third at 0°C without puromycin. Similar experiments were performed with [^{3}H]galactose (250 μCi per ml): one piece of fundus was incubated at 37°C, another at 0°C. The pieces were fixed in the OsO_4-fixative as used for electron microscope autoradiography and embedded in paraffin, and 5-μm sections were covered by stripping film (Kodak AR10). Exposure time was 6 days, to obtain an effective dose from the sections of pieces of fundus incubated at 37°C, equal to the dose radiated from the EM sections during a 60-day exposure. After development, silver grains were counted over areas of surface epithelium containing about 100 nuclei. The number of silver grains divided by the number of nuclei was used as a measure of the radioactivity per cell. Relative to the amount of radioactivity found after incubation at 37°C, aspecifically bound radioactivity amounted to 6.2% (0°C incorporation) or 9.8% (puromycin) for leucine, and to 10.2% (0°C) for galactose.

These data are somewhat too high, since a certain amount of radioactivity is incorporated even in the presence of puromycin or at 0°C.

RESULTS

Ultrastructure

The ultrastructure of the SMC of the rat as observed in this study does not differ from that of earlier descriptions (5, 16, 38). Therefore, the description here is confined to those elements that play a role in the synthesis and the transport of the secretory product (see Fig. 5).

The RER of the SMC is not extensive. RER cisternae are small and contain a flocculent material. Free ribosomes are present throughout the cytoplasm but mainly in the apical part. The Golgi stacks lie close to the nucleus, their mature cisternae facing the apex and lateral sides of the nucleus. The nucleus is frequently indented by condensing vacuoles. The Golgi complex consists of 3–6 stacked cisternae with many smooth vesicles and tubules at the immature side. Vesicles, some of which are coated, are also present at the mature side. Although many mature granules, which often have an electron-opaque core, fill the top of the cell, signs of exocytotic secretion are rare. Scattered throughout the cytoplasm between the golgi cisternae and the mass of mature granules are young granules that are denser than the condensing vacuoles. Bundles of filaments (Figs. 6 and 7) lie at the lateral sides of the cells beneath the mass of packed mature granules.

Incubation apparently causes the connective tissue of the lamina propria to retract from the epithelium. In light microscope preparations, the epithelium seems to bridge the empty spaces between the gastric pits. This results in a decreased number of collagen fibrils (Fig. 6). In some cases, the retraction of the lamina propria starts within the first hours of incubation.

The ultrastructure of the SMC does not show significant alterations after incubation for up to 4 h (Fig. 6). After longer times, the base of the cells becomes smaller and the intercellular spaces remarkably wider. Some cells show signs of vacuolization and autophagocytosis, though normal cells are present, even after 6 h of incubation.

The volume densities of the three main intracellular compartments involved in (glyco)protein transport are rather constant over the 2-h period measured. They amount to 15.3% (SD = 2.2%) for the RER, to 13.5% (±0.7%) for the Golgi complex (including condensing vacuoles and youngest secretory granules), and to 12.6% (±2.5%) for the mucous granules.

Transport of Incorporated [^{3}H]Leucine

Since the rate of incorporation of [^{3}H]leucine into the SMC is low and results in a small yield of autoradiographic silver grains, many cell sections had to be screened. Table II shows the results of the countings of silver grains over SMC of the surface epithelium and upper half of the gastric pits.

Five minutes after the start of pulse labeling, silver grains are present over a variety of cellular structures such as nuclei, mitochondria, and filaments (Fig. 7). At this time, the RER shows its highest radioactivity, which decreases gradually during postincubation. The Golgi complex shows a high radioactivity between 10 and 60 min with a peak around 40 min. Condensing vacuoles and the youngest secretory granules are maximally labeled at 60 min and mature granules not earlier than 2 h (Fig. 8). Two hours after pulse labeling, silver grains are found in the mucus layer that adheres to the cells (Fig. 8). The changes in relative concentration of silver grains over transport structures of the SMC after leucine incorporation are shown in Fig. 11 A.

Transport of Incorporated [^{3}H]Galactose

Shortly after a 3-min pulse labeling, the Golgi complex shows the highest radioactivity (Fig. 9), which decreases gradually with postincubation time. The radioactivity of condensing vacuoles and young secretory granules peaks at 10 min. Mucous granules become radioactive at 40 min (Fig. 10). Radioactivity in the RER is low, even shortly after pulse labeling. In the mucus layer, radioactivity is found 60 min after pulse labeling.

The changes in the relative concentration of silver grains over transport structures of the SMC after galactose incorporation are shown in Fig. 11 B. Again, as in the case of labeling with leucine, the data on relative concentrations do not substantially alter the conclusions taken from the relative grain distributions. Apparently, the selection of micrographs for quantitative autoradiography was sufficiently random.

DISCUSSION

The preliminary experiments show convincingly

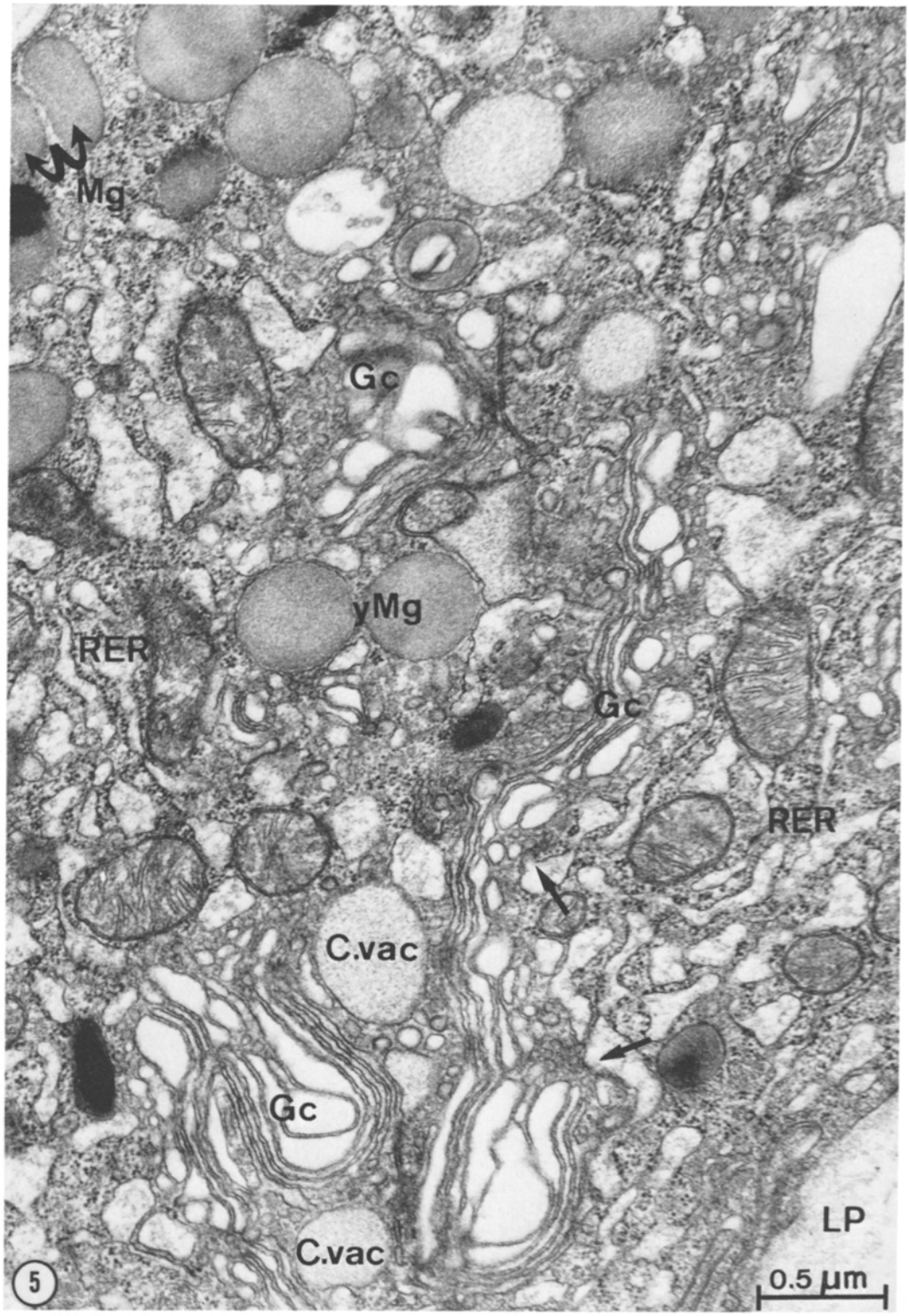


FIGURE 5 Part of an unincubated SMC of the rat fundus, showing RER cisternae containing floccular material (*RER*); transitional elements of the RER (arrows) at the immature side of the Golgi complex (*Gc*); a condensing vacuole (*C. vac*) at the mature side; young (*yMg*) and mature (*Mg*) mucous secretory granules. *LP:* lamina propria. Note the vesicular and tubular elements at both sides of the Golgi stacks. × 39,000.

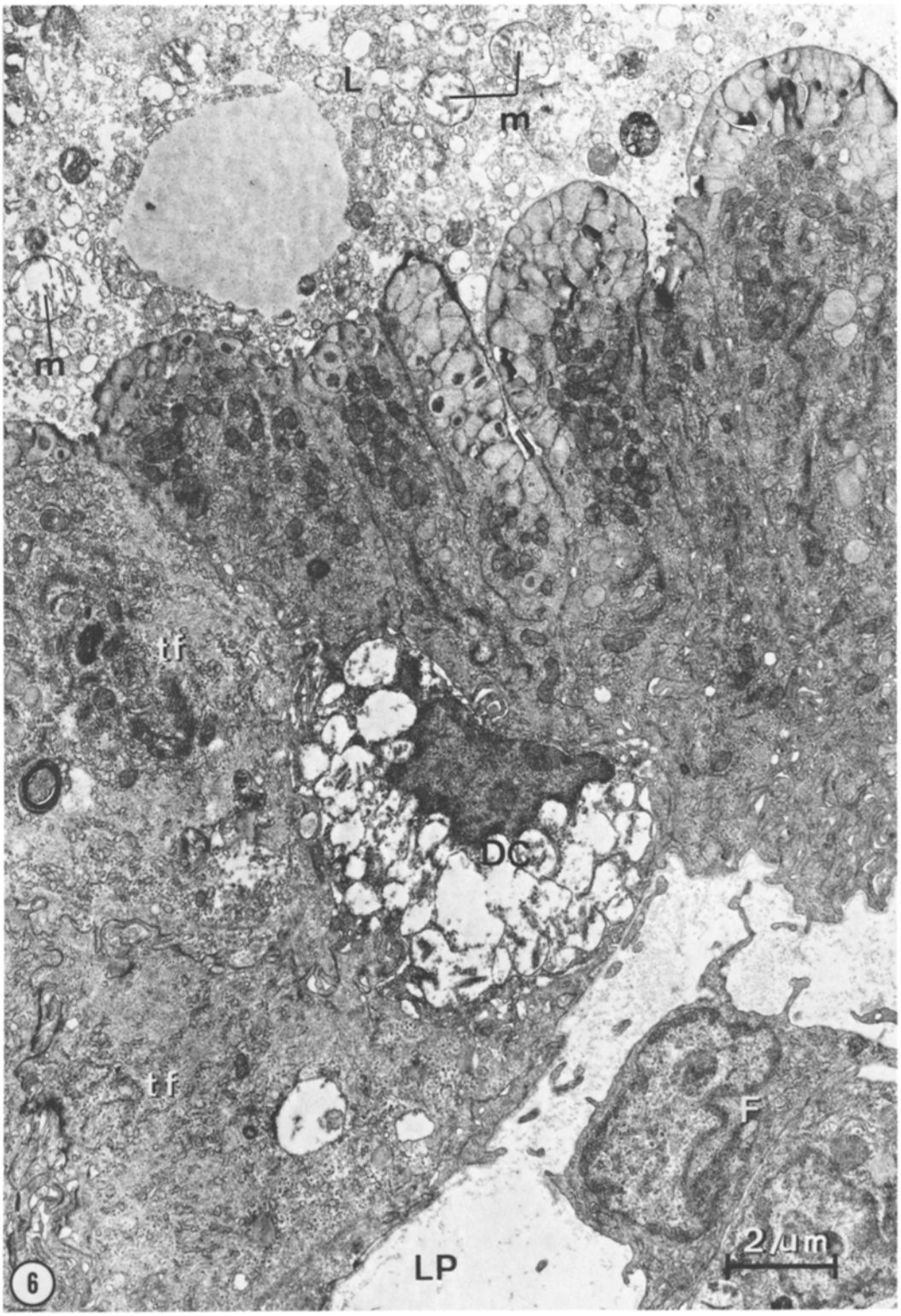
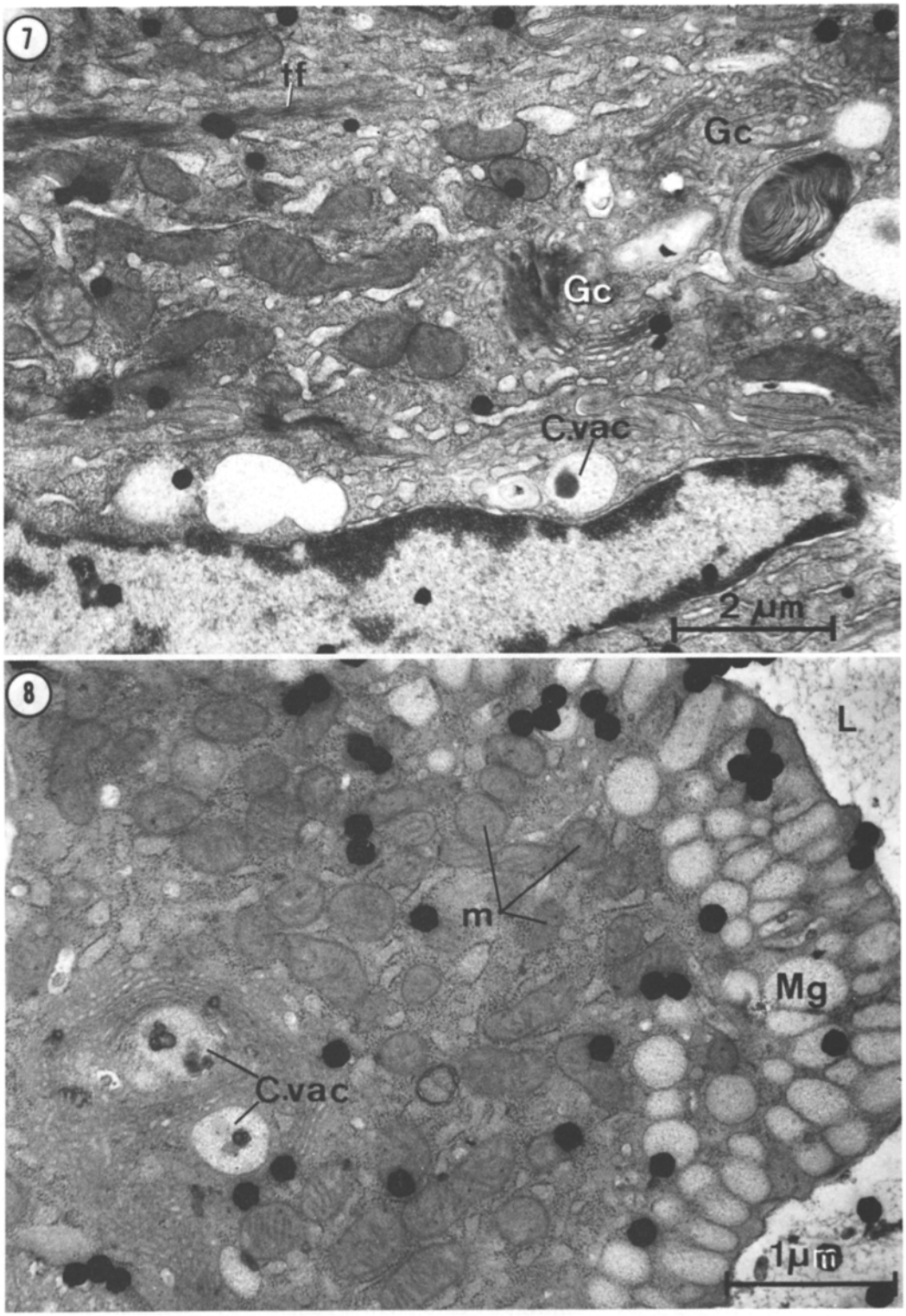


FIGURE 6 Surface lining of rat fundus, incubated for 4 h. Surface mucous cells bordering the lumen (*L*) have a normal appearance. The type of degenerating cell (*DC*) present in this area is a normal feature of gastric epithelium. The lumen contains cell debris mainly consisting of parietal cell mitochondria (*m*). The lamina propria (*LP*) shows a reduced number of collagen fibrils. *F*: fibroblast; *tf*: tonofilaments. × 7,900.



FIGURES 7–10 EM autoradiographs of SMC of the rat fundus, labeled by [^{3}H]leucine or [^{3}H]galactose. The autoradiographs were exposed for 6 mo and developed in Phenidone (for details, see text). *Gc*: Golgi complex; *C. vac*: condensing vacuoles; *yMg*: young mucous secretory granules; *Mg*: mature mucous secretory granules; *m*: mitochondria; *tf*: tonofilaments; *L*: lumen.

FIGURE 7 [^{3}H]leucine (3 + 2 min). Label is found throughout the cell over the RER, Golgi complex, condensing vacuoles, nucleus and tonofilaments. × 12,000.

FIGURE 8 [^{3}H]leucine (5 + 115 min). Silver grains are mainly associated with mucous granules. Grains present over the lumen indicate secretion. × 26,000.

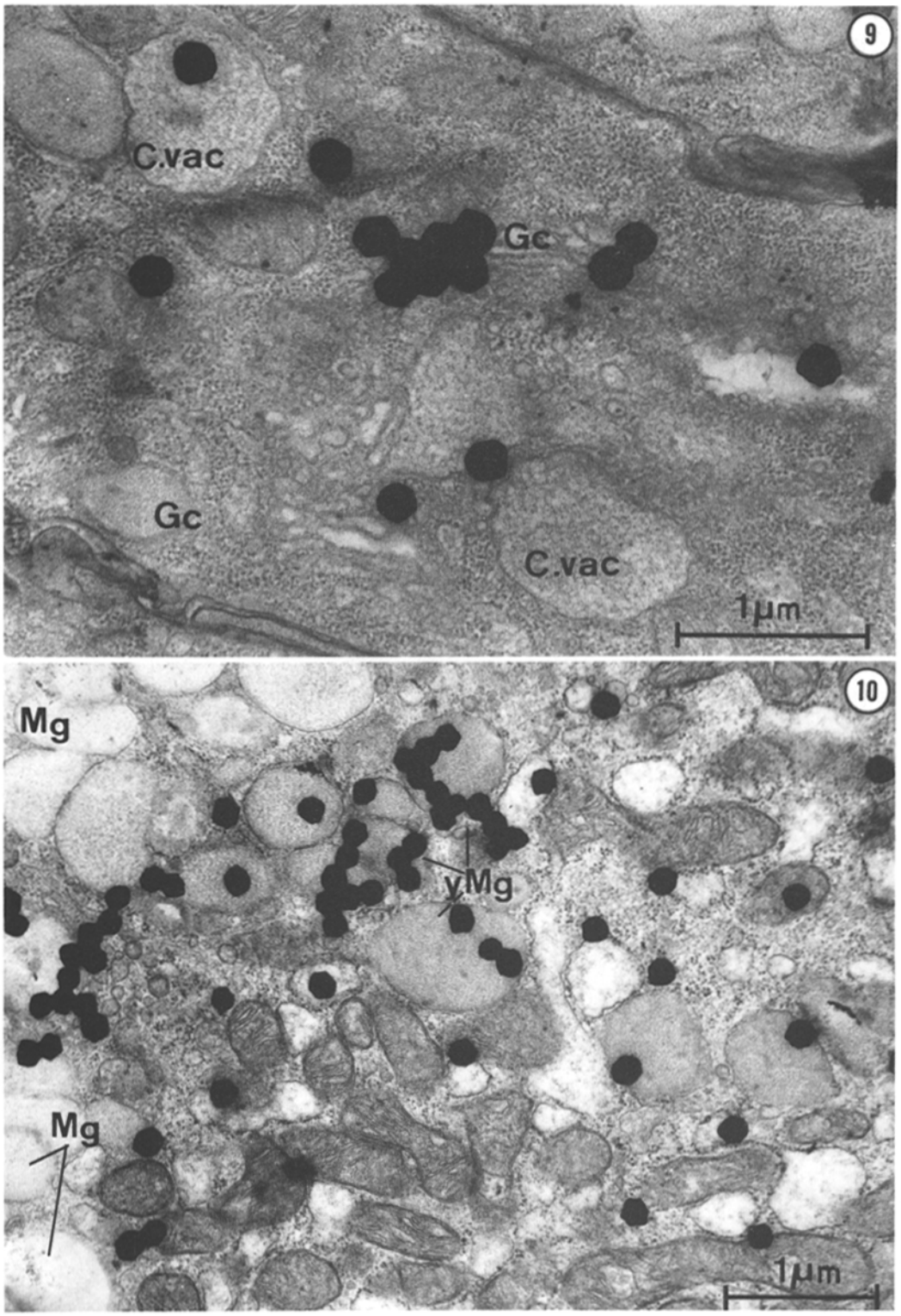


FIGURE 9 [^{3}H]galactose (3 + 2 min). Silver grains are mainly associated with Golgi complex and condensing vacuoles. × 29,000.

FIGURE 10 [^{3}H]galactose (5 + 35 min). Silver grains are concentrated over the young mucous secretory granules. × 23,000.

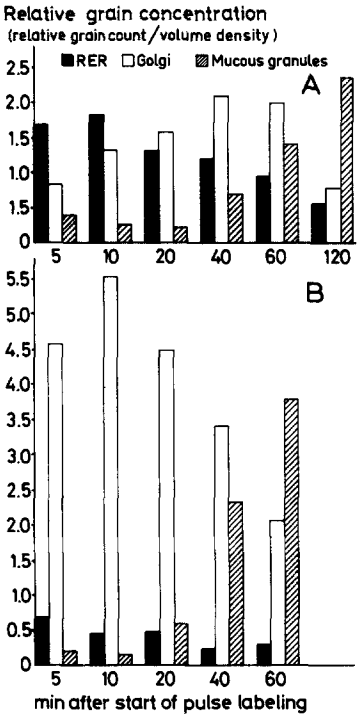


FIGURE 11 Relative silver grain concentration over RER, Golgi system (including condensing vacuoles and youngest secretory granules), and mucous granules at different times after pulse labeling with: (A) [^{3}H]leucine, and (B) [^{3}H]galactose.

that the SMC of the fundus of the rat stomach survive in vitro conditions for up to 6 h. After 6 h, some of the SMC show ultrastructural signs of degeneration, i.e., vacuolization and autophagocytosis, but in the isthmus and the lower part of the gastric pits mitotic activity remains normal as demonstrated by the incorporation of labeled thymidine. The rate of incorporation of leucine decreases after 4 h, but that of galactose is the same as at the start of the incubation.

The structure of the fundic mucosa is damaged earlier. Even after 1 h of incubation, there are a retraction of the connective tissue of the lamina propria and a loss of parietal cells. At later times, the connection between pits and glands is sometimes disrupted, and after 6 h many pepsinogen cells have lost their stainability by pyronin, which indicates ribosomal RNA breakdown.

Because of the connective tissue retraction, the SMC layer spans the "empty" spaces between the pits. The displacement of the fibrillar and interfibrillar elements of the connective tissue might result from contraction of the connective tissue itself or from the uptake of fluid (mucosal edema). The flattening of the SMC at the longer incubation times suggests a gradual loss of cells or an increase in surface area as might result from edema. However, little labeled (glyco)protein reaches the medium within 6 h after pulse labeling with tritiated leucine in vitro. Furthermore, labeled cell residues have not been observed in the mucous layer adhering to the epithelium.

We used mucosal tissue fragments because of their long survival in vitro and their suitability for a autoradiographic study of glycoprotein transport in cells that can be identified as to type and localization. In other studies, scrapings of gastric mucosa were used. Human antral scrapings incorporate glucose, but oxygen consumption decreases during 3.5 h of incubation (13). Scrapings of fundic mucosa appeared to incorporate leucine for only 5–15 min (12). Pig gastric mucosal scrapings appeared to survive for about 2 h, and to incorporate glucose, threonine, and serine during that time (33).

In this study, the secretory product of the SMC is supposed to consist mainly of glycoproteins. Pepsinogens are not secretory products of these cells (29, 30, 41, 43). In the rat, intrinsic factor is produced by the pepsinogen cells (9). A possible product of the SMC is the gastric secretion-inhibiting substance, gastrone, but this is also a nondialysable glycopeptide (7).

It was also assumed that the glycoproteins produced and secreted by the SMC contain galactose residues. This has been shown for gastric mucous glycoproteins of man (31) and pig (32), but not for rat.

The intracellular transport of the protein moiety of the secretory product of the SMC is similar to that in other secretory cells. The rate of transport from the RER to the Golgi system is nearly the same as in the rabbit parotid gland cells in vitro (4) and slower than in the exocrine cells of the guinea pig pancreas (11) and the somatotrophs (10) and mammotrophs (17) of the rat adenohypophysis in vitro. In our study the radioactivity of the Golgi system peaks later than in any other cell type studied in vitro (4, 10, 11, 17).

In SMC, labeled galactose is mainly incorporated into the Golgi area. Two minutes after a 3-min pulse labeling in vitro, 47% of the silver grains lie above Golgi vesicles, tubules, and cisternae. In thyroid (40) and parathyroid cells (22), the relative amount of galactose incorporated into the Golgi elements is about 60%, and in amelo-

blasts, 75% (39). Condensing vacuoles are not so clearly defined in these cell types as in the SMC, and some of the silver grains may have lain over these structures. In the SMC, 72% of the silver grains lie over Golgi elements and condensing vacuoles. Other cell types too have been shown, though not quantitatively, to incorporate galactose mainly into the Golgi complex (8, 23, 25, 36, 42).

From the present autoradiographic study it is clear that in the SMC most of the galactosyl transferase activity is in the Golgi complex. Galactosyl transferase has been localized in the Golgi complex of the pancreatic cells of the guinea pig (20), and in that of spermatocytes and spermatids (6), sublingual and submandibular gland cells (35), and liver cells of the rat (2, 24). Galactosyl transferase is also present in the microsome fraction of gastric mucosa of man, the baboon, and the rabbit (26, 44) and has been demonstrated in rat SMC microsomes, too (34).

Within half an hour, 55% (intrapolated) of the galactose radioactivity has left the Golgi complex of the SMC, while 58% is lost from thyroid cells (40) at that time. On the other hand, under in vivo conditions, ameloblasts (39), parathyroid cells (22), and hepatocytes (21) have lost at least 75% from their Golgi complex.

15% of the galactose incorporated into the SMC is in condensing vacuoles and secretory granules 2 min after a 3-min pulse. The rapid appearance of labeled glycoprotein in these structures might be due to the attachment of galactose to the growing chains of carbohydrates shortly before the glycoprotein is packed into condensing vacuoles. In rat liver cells, galactosyl transferase is found mainly in a Golgi subfraction, characterized by the presence of secretory vacuoles (24). This is in accordance with the hypothetical high concentration of galactosyl transferase at the mature side of the Golgi complex. However, in another study (2) the enzyme appeared to be in a Golgi subfraction mainly composed of immature elements. The rate of glycoprotein transport from the Golgi complex to the condensing vacuoles and secretory granules is reflected by the loss of radioactivity from the Golgi complex. This loss is more rapid after galactose than after leucine labeling in the SMC (Fig. 12 A) and in parathyroid cells (22). Since the loss of leucine from the Golgi complex is influenced by the slow transfer of leucine radioactivity from the RER into the Golgi complex, galactose radioactivity is more indicative of the transport rate from the Golgi area.

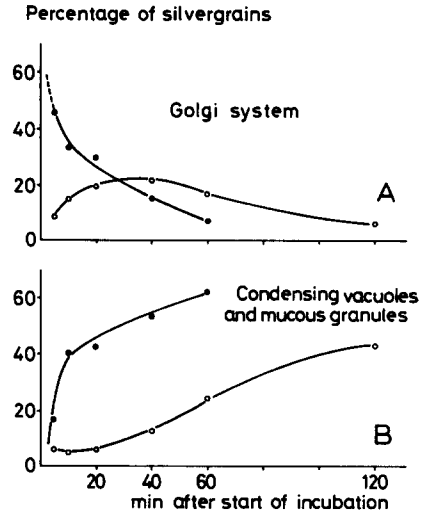


FIGURE 12 The relative number of silver grains over (A) the Golgi system and (B) condensing vacuoles and mucous secretory granules at different times after the start of incubation for 3 or 5 min with [^{3}H]leucine (○——○), or [^{3}H]galactose (●——●).

The arrival of galactose-labeled glycoprotein into the condensing vacuoles is biphasic (Fig. 12 B). The first phase may represent glycoprotein molecules with galactose residues attaching within the mature side of the Golgi complex. If membrane glycoproteins reach the condensing vacuoles at a slower rate than the secretory glycoproteins, this could cause the second phase in the transport of galactose-labeled protein from the Golgi complex, as suggested by Riordan et al. (27) for liver cells.

The relative concentration of radioactivity in nuclei, mitochondria, and the remainder of the cytoplasm together is very high and constant at all intervals after [^{3}H]leucine incorporation, and low and constant after [^{3}H]galactose incorporation (0.79 ± 0.09 and 0.39 ± 0.05). In view of the low background and small amount of aspecific binding of the precursors, the high leucine value suggests that a great part of the radioactive amino acid is incorporated into constitutive protein. The value of 46%, suggested by Table II, is too high, since it has not been corrected for aspecific binding and cross firing from "transport" compartments. Nevertheless, it is higher than in any other secretory cell studied (3, 4, 10, 11, 17, 28, 40). This is in good agreement with the large number of free ribosomes and the rather low amount of RER in the SMC (15% of the cell volume). The presence of radioactivity in the nuclei, mitochondria, and

cytoplasm after galactose incorporation might also be explained by transfer of the precursor to constitutive glycoproteins.

The presence of radioactive (glyco)protein outside the "transport" compartments might also be interpreted as an indication of cytoplasmic transport. However, the constancy of the relative concentration of radioactivity outside the compartments, while the concentration changes within them, is difficult to reconcile with a cytoplasmic transport of secretory product from one compartment to another at a given time after its labeling, and also with an equilibrated exchange between each of the compartments and the matrix (unless all compartments have the same permeability and binding constants). The constancy does not exclude a direct release of leucine-labeled protein from the membrane-bound ribosomes and of galactose-labeled glycoprotein from the Golgi cisternae towards the cytoplasm, from where they should start to be secreted after more than 60 and 40 min, respectively.

In vitro, no radioactive glycoprotein is found in the medium within 4 h after [^{3}H]galactose administration. In the electron microscope autoradiographs, however, radioactivity is demonstrated in the mucus layer on the SMC after 60 min. This suggests that the glycoprotein is secreted in a nondissolved form. Apparently, the secreted glycoproteins stick to the mucus layer before being dissolved.

The glycoprotein secreted by the SMC is part of the gastric content and may aid in protecting the gastric wall against the digestive action of pepsin and acid. It will be interesting to further study glycoprotein transport and secretion under stimulated and pathological conditions. The characteristics of the transport in vitro reported here offer a base for such a comparative study.

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