

Molecular Organization of Prolactin Granules

III. Intracellular Transport of Sulfated Glycosaminoglycans and Glycoproteins of the Bovine Prolactin Granule Matrix

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ABSTRACT The intracellular transport of sulfated glycosaminoglycans (heparan sulfate and chondroitin sulfate) and glycoproteins of the prolactin (PRL) granule matrix, as well as that of PRL, was studied using a system of double-labeled bovine anterior pituitary slices. [³⁵S]sulfate was used to label sulfated macromolecules and L-[³H]leucine to label PRL. In membraneless granules (isolated from a PRL granule fraction after solubilization of the membrane with Lubrol PX), sulfated glycosaminoglycans and glycoproteins were considerably labeled after a 15-min pulse, while the hormone was still unlabeled. During the chase incubation, the specific radioactivity of granule PRL and of the various complex carbohydrate classes first increased, reaching a peak after ~40 min, and then began to decline. After 4 h of chase incubation the radioactivity remaining in granule PRL and sulfated complex carbohydrates was 50–60% of that observed at 40 min. Thus, in pituitary mammothrophs a pool of sulfated glycoproteins and glycosaminoglycans is transported intracellularly in parallel with PRL. This finding corroborates the previous conclusion (Zanini et al., 1980, *J. Cell. Biol.* 86:260–272) that sulfated macromolecules are structural components of the granule matrix. The discharge of labeled PRL and complex carbohydrates from the slices to the incubation medium was also investigated. [³⁵S]-glycosaminoglycans and glycoproteins were released at a rapid rate during the first 30–40 min of chase incubation, when PRL granules had not yet attained maximum specific activities. By 40 min, their release tended to level off but the radioactivity accumulating in the incubation medium was still much larger (approximately a fourfold increase) than the losses observed concomitantly in PRL granules. These discharge kinetics contrast with that of [³H]PRL, which was not released during the 1st h of chase incubation but then began to accumulate at a high rate in the medium, in parallel with its decrease in granules. Dopamine (5×10^{-7} M) strongly inhibited the release of labeled PRL but had no detectable effect on the release of labeled glycosaminoglycans and glycoproteins or on the discharge of ³⁵S-macromolecules as revealed by SDS polyacrylamide gel electrophoresis of incubation media. Thus the releases of PRL and sulfated macromolecules have different kinetics and can be dissociated from each other. These data indicate that much of the glycosaminoglycans and glycoproteins released from pituitary slices originates from sites other than PRL granules, and that at least part of the complex carbohydrates of the PRL granule matrix might not be released with the hormone but rather remains associated with the mammothroph cells after exocytosis.

In the preceding article (32) we have reported extensive biochemical evidence indicating that in bovine pituitary mam-

mothrophs small amounts of glycosaminoglycans and glycoproteins (part of which are sulfated) are present together with the

specific hormone prolactin (PRL)¹ in the matrix of secretory granules. This report concerns the intracellular movement of these sulfated macromolecules, which was investigated by means of pulse-chase experiments. Specifically, we have compared the intracellular transport, and release to the extracellular medium, of PRL and the various sulfated macromolecules using bovine pituitary slices doubly labeled with [³⁵S]sulfate and L-[³H]leucine.

At the beginning of these studies it was anticipated that the results obtained might be of value in understanding the functional role of sulfated macromolecules in the granule matrix, particularly in relation to the possibility of their participation in the packaging of secretion products (as suggested previously for other secretory systems [21, 28, 29]). If the function of these molecules were in the formation of large aggregates formed by direct binding to PRL molecules, they would be expected to be transported through the cell and discharged into the extracellular space concomitantly with the hormone. Conversely, differences in the intracellular route and time course as well as in the final disposition of PRL and sulfated macromolecules, might be of help in identifying a specific function for the latter molecules in mammoth cells.

MATERIALS AND METHODS

In Vitro Incubation

Bovine pituitary slices, prepared from two to three pituitary glands, as described in the preceding report (32) were transferred to 100-ml Erlenmeyer flasks containing 15–20 ml of pulse medium consisting of oxygenated Krebs-Ringer-bicarbonate buffer (KRB) with added glucose and amino acids (except leucine), and in which MgSO₄ had been replaced by 1.14 mM MgCl₂, together with [³⁵S]sulfate (225–325 μ Ci/ml; sp act: 160–640 mCi/ μ mol) and L-[³H]leucine (150 μ Ci/ml; sp act: 1 mCi/ μ mol). Incubation in this radioactive medium was carried out under 95% O₂ + 5% CO₂ for 10 min at 0°C followed by 15 min at 37°C in a shaking bath operated at 60 cycles/min. The slices were then separated from the incubation medium by filtration through nylon mesh, and rinsed thoroughly with large volumes of warm, unlabeled KRB containing 1.14 mM MgSO₄ and 2 mM leucine (chase medium). About one-fifth of the slices was set aside and the rest was divided into four similar sets which were reincubated in 30 ml of chase medium at 37°C under 95% O₂ + 5% CO₂ for 15, 40, 90, and 240 min.

In experiments designed to investigate the effect of dopamine on the release of sulfated glycosaminoglycans and glycoproteins, the pituitary slices labeled with [³⁵S]sulfate and L-[³H]leucine were rinsed carefully with warm chase medium (3 \times 2 min changes) and then reincubated in chase medium containing various concentrations of the catecholamine (10⁻⁵, 5 \times 10⁻⁶, 5 \times 10⁻⁷, 5 \times 10⁻⁸ M). This chase incubation was continued for a total of 180 min, with changes of medium every 30 min.

At the end of the incubation the slices were rinsed with ice-cold 0.32 M sucrose and homogenized. The media were centrifuged (100,000 g, 60 min) to remove tissue fragments and cell debris, and then extensively dialysed.

Cell Fractionation and Biochemical Methods

Homogenates obtained from incubated pituitary slices were fractionated by differential and density gradient centrifugation as described in the preceding article (32). The postnuclear supernate, total microsomes, and membraneless PRL granules (MLG) were used for the present studies. The techniques used for the purification of growth hormone (GH), PRL, glycosaminoglycans and glycoproteins; for measuring radioactivity in total protein, total sulfated macromolecules, GH, PRL, glycosaminoglycans and glycoproteins; for SDS polyacrylamide gel electrophoresis (PAGE) and for protein assay are described in the preceding article (32).

¹ *Abbreviations used in this paper:* ER, endoplasmic reticulum; GH, growth hormone; KRB, Krebs-Ringer-bicarbonate; MLG, membraneless prolactin granules; PAGE, polyacrylamide gel electrophoresis; PRL, prolactin.

Materials

Dopamine was purchased from Sigma Chemical Co. (St. Louis, Mo.). Other materials were obtained from the sources specified in the preceding article (32).

RESULTS

Bovine pituitary slices incubated with L-[³H]leucine and [³⁵S]sulfate incorporated these two precursors into macromolecules at a linear rate for several hours. When the slices, doubly labeled for 15 min, were transferred to chase medium, this incorporation rapidly ceased, as shown by the fact that the total macromolecular ³H and ³⁵S radioactivity remained constant in the tissue + incubation medium over a period of 4 h. These findings indicate that, as reported previously for leucine (18), the intra- and extracellular pools of sulfate equilibrate readily in pituitary slices. Thus, changes in the radioactivity of sulfated macromolecules occurring in pituitary organelles during chase incubation cannot be accounted for by ongoing incorporation of the labeled precursor, but must be attributed to metabolism and/or redistribution (intracellular transport and discharge).

Intracellular Transport

The time-course of labeling of PRL and of the total sulfated macromolecules during chase incubation was followed in the microsomes and MLG. The corresponding sulfate labeling of chondroitin sulfate, heparan sulfate, and glycoproteins was also determined in the MLG. As can be seen in Figs. 1 and 2, at the end of the pulse labeling both radioactive PRL and sulfated macromolecules were highly concentrated in the microsomal fraction, with levels exceeding those of the postnuclear supernate by factors of ~3 and 5, respectively.² In MLG the PRL was still unlabeled at this time-point, although there was already considerable sulfate labeling (~44% of that in the postnuclear supernate). Approximately 56% of the MLG sulfate radioactivity was in glycoproteins, 26% in heparan sulfate, and 18% in chondroitin sulfate.

After 15 min of chase incubation the PRL radioactivity in microsomes was reduced to 66% and the ³⁵S radioactivity to <50%. Concomitantly, radioactive PRL began to accumulate in MLG, where the labeling of sulfated glycoproteins and glycosaminoglycans was also considerably increased. Between 15 and 40 min there was a further large decrease in PRL radioactivity in microsomes, while the decrease of ³⁵S macromolecules was more moderate. Thereafter the two labels continued to decrease in microsomes at much slower rates. In MLG the radioactivity of PRL, glycosaminoglycans, and glycoproteins increased until 40 min of chase incubation and then began to decline. Initially (until 90 min) the rate of decrease was faster for the sulfated macromolecules, whereas later the decreases were at approximately the same rate for the two labels. After 240 min of chase incubation the radioactivity remaining in glycosaminoglycans, glycoproteins, and PRL was between 50 and 60% of the peak attained at 40 min (Figs. 1 and 2).

² The different relative enrichment of labeled PRL and complex carbohydrates in the microsomal fraction at the end of the pulse might be caused by several factors, such as different recoveries of the cellular elements where the two labels are located (ER and Golgi complex), or differences in the leakage and reabsorption of the segregated molecules from and onto these elements during tissue homogenization and fractionation (see reference 27).

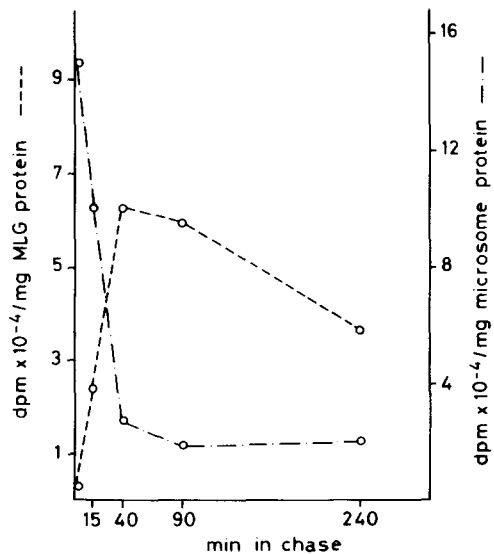


FIGURE 1 Time-course of L-[³H]leucine incorporation into PRL of microsomes (○---○) and MLG (○---○). The fractions were isolated from pituitary slices pulse-labeled with L-[³H]leucine and [³⁵S]inorganic sulfate for 15 min, and then incubated in chase medium for the time indicated on the abscissa. The values given are the averages of three experiments.

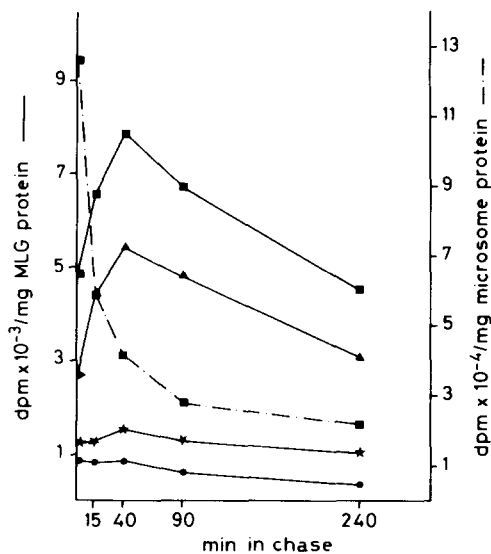


FIGURE 2 Time-course of ³⁵S incorporation into total sulfated macromolecules of microsomes (■---■) and MLG (■---■), as well as into glycoproteins (▲---▲), heparan sulfate (★---★), and chondroitin sulfate (●---●) of MLG. The fractions were isolated from pituitary slices doubly labeled with L-[³H]leucine and [³⁵S]inorganic sulfate, and then incubated with chase medium for the times indicated on the abscissa. Values given are the averages of two experiments.

Discharge to the Incubation Medium

The release kinetics of ³⁵S-labeled complex carbohydrates and ³H-labeled hormones (PRL and GH) into the incubation medium is shown in Fig. 3. A clear difference between these two classes of macromolecules is immediately apparent. Release of ³⁵S-labeled glycosaminoglycans and glycoproteins occurred at a rapid rate during the first 40 min of chase incubation and then began to decline. By 240 min, approximately half of the ³⁵S-labeled macromolecules originally present in the tissue had been discharged into the medium. In contrast, there was

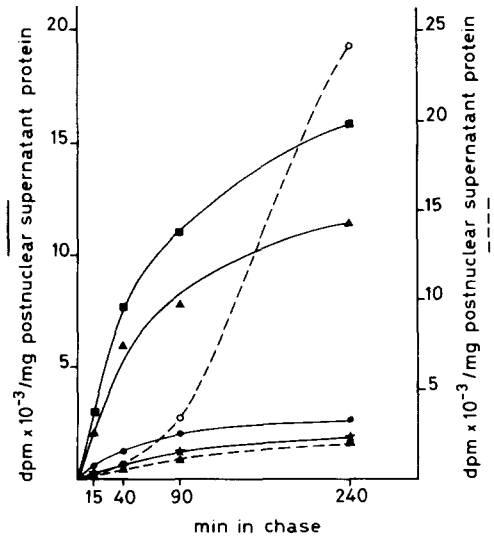


FIGURE 3 Kinetics of discharge to the incubation medium of ³H-labeled GH (▲---▲) and PRL (○---○), and ³⁵S-labeled total sulfated macromolecules (■---■), glycoproteins (▲---▲), chondroitin sulfate (●---●), and heparan sulfate (★---★). Pituitary slices were labeled with L-[³H]leucine and [³⁵S]inorganic sulfate, divided into five sets, and incubated in chase medium. At the times indicated on the abscissa a set of slices was separated from the medium by filtration, the latter was centrifuged at high speed, and the resulting supernate was analyzed. Values given are the averages of two experiments.

little release of ³H-labeled PRL until 60 min of chase incubation. This lag represents the time required for intracellular transport of PRL from the endoplasmic reticulum (ER) to mature secretion granules (11, 18). The release of GH occurred at a rate much slower than that of PRL.

The data in Fig. 3 were compared with those in Figs. 1 and 2, recalculated on the basis of postnuclear supernate protein, and corrected for the incomplete recovery of PRL granule matrix in the MLG fraction.³ We found that the amount of PRL radioactivity which disappeared from the granules during the 40- to 240-min interval of chase incubation represents a substantial portion (~65%) of that appearing in the medium. This finding is consistent with the widely accepted view that PRL release occurs exclusively by exocytosis of mammoth granules (8, 10, 11). In fact, the small discrepancy between granule loss and medium gain might be caused by the delayed transport of some labeled hormone, as a consequence of a small degree of intermixing with nonradioactive hormone within the ER and Golgi cisternae (11). On the other hand, the situation observed with sulfated macromolecules was entirely different. The rate of release of ³⁵S macromolecules was most rapid during the first 40 min of chase incubation, at times before the PRL granules were maximally labeled. Moreover, even during the 40- to 240-min chase period, the amounts of sulfate radioactivity released into the medium greatly exceeded the amounts lost by PRL granules (by approximately 3-, 4-, and 6.5-fold for heparan sulfate, sulfated glycoproteins, and chondroitin sulfate, respectively). There was also a clear difference in the distribution of radioactivity between the two sul-

³ For this correction we assume that, in the tissue, PRL granules account for the entire hormone content minus the fraction present in the ER and Golgi complex. The latter was found to represent 5-10% of the total, calculated from the data on microsomes and corrected for incomplete recovery with the aid of marker enzymes. The granule contribution was therefore taken as 90% of the total tissue store.

fated glycosaminoglycans, because in MLG more labeling was present in heparan sulfate than in chondroitin sulfate, while the reverse was found in the medium.

Further information on the observed nonparallelism in labeled PRL and sulfated macromolecule release was sought by studying the effect of dopamine. We confirmed that at concentrations between 5×10^{-7} and 10^{-5} M, dopamine inhibits the *in vitro* release of PRL by ~90%. The effect of dopamine (5×10^{-7} M) on the release of sulfated macromolecules was then studied (three separate experiments). In the experiment illustrated in Fig. 4 and Table I, we followed the release of [3 H]-PRL and [35 S]glycosaminoglycans and glycoproteins from slices which had been prelabeled for 15 min. Under these conditions, the dopamine inhibition of [3 H]PRL release appeared after the time required for intracellular transport (i.e., during the 2nd and 3rd h of chase incubation). However, experiments carried out using longer labeling times (not shown) as well as extensive evidence from different animal species (7, 16) indicate that the inhibition of PRL release develops immediately after addition of dopamine. In contrast to the results obtained with PRL, the addition of dopamine to the chase medium had no consistent effect on the release of either total nondialyzable 35 S radioactivity (Fig. 4) or individual labeled sulfated glycoconjugates (Table I) at any of the time-points investigated.

The products released during chase incubation were also analyzed by SDS PAGE. The distribution pattern of 35 S-radioactivity is illustrated in Fig. 5. A considerable portion of the counts present in the early chase media (0-30, not shown, and 30-60 min of chase incubation, Fig. 5A) was recovered in a large peak migrating between PRL and GH, as well as in the high molecular weight region of the gel. During the following time periods significant changes in this pattern were observed. The large early peak and the counts recovered in the high molecular weight region were considerably reduced, whereas two other peaks (one migrating ahead of GH and another corresponding to an apparent molecular weight of ~70,000)

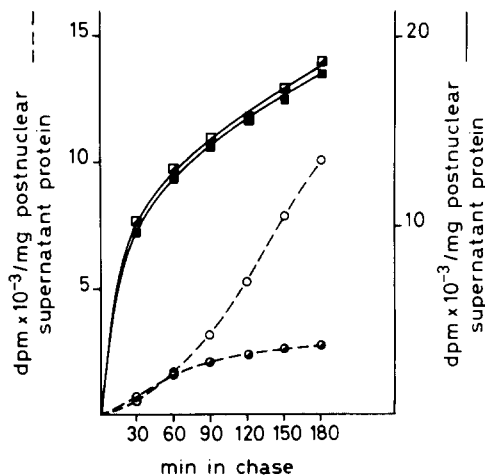


FIGURE 4 Effects of dopamine on the kinetics of discharge to the incubation medium of 3 H-labeled PRL and 35 S-labeled total sulfated macromolecules. Pituitary slices were labeled with L-[3 H]leucine and [35 S]inorganic sulfate for 15 min, washed with warm chase medium (3×2 min washes) and incubated in the latter, with or without 5×10^{-7} M dopamine, for a total of 180 min. The medium was changed every 30 min, centrifuged at high speed, and the supernate analyzed. \circ - \circ - \circ and \bullet - \bullet - \bullet : PRL, - and + dopamine; \blacksquare - \blacksquare - \blacksquare and \square - \square - \square : total sulfated macromolecules, - and + dopamine.

TABLE I
Effect of Dopamine (5×10^{-7} M) on the Release of 35 S-labeled Glycoproteins, Heparan Sulfate, and Chondroitin Sulfate from Bovine Pituitary Slices

Incubation medium	min In chase	Glycoproteins	Heparan sulfate	Chondroitin sulfate
KRB	0-60	1,114,918	44,285	67,605
Dopamine	0-60	1,135,238	42,432	56,675
KRB	60-120	264,404	12,900	13,908
Dopamine	60-120	232,236	11,327	13,402

Data from the experiment illustrated in Figs. 4 and 5. Equal aliquots of the centrifuged media collected at 30 and 60 min as well as at 90 and 120 min of chase incubation were pooled and analyzed. Values are expressed as dpm/100 mg protein in slices at the end of the incubation.

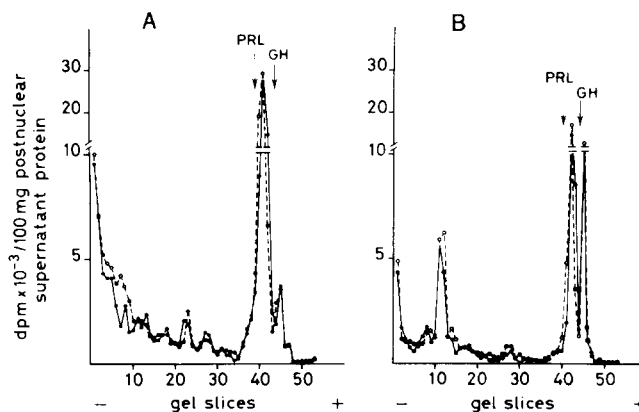


FIGURE 5 SDS-10% PAGE of the 35 S-labeled macromolecules discharged to the medium during 30-60 (A) and 120-150 (B) min of chase incubation, with or without dopamine. Same experiment as that illustrated in Fig. 4. The position of [3 H]PRL and [3 H]GH in the gel is indicated by arrows. No dopamine, \bullet - \bullet ; 5×10^{-7} M dopamine \circ - \circ .

were increased (Fig. 5B). No significant differences between the control and dopamine patterns were revealed in any of the media investigated.

DISCUSSION

Much of the previous information on the intracellular transport of sulfated macromolecules came from electron microscope radioautography studies. By this experimental approach it was shown that in various cell systems, including the acinar cells of the exocrine pancreas and parotid gland (1-3, 25, 30) and, recently, the rat pituitary mammatroph and somatotroph cells (26), [35 S]sulfate residues are incorporated into macromolecules in one single subcellular structure, the Golgi complex. 35 S macromolecules then move from the Golgi complex to secretion granules, and possibly also to other sites within the cell (1-3). The later decrease of radioactivity in the granule compartment was often interpreted as an indication that sulfated macromolecules are discharged to the extracellular space, together with the secretion products segregated in the granule contents (1, 2, 15).

The results obtained by radioautography are of unique interest because they refer to individual subcellular structures within well-identified cell types. However, the radioautographic approach does not yield information on the quantity and chemical nature of the sulfated macromolecules transported along the secretory pathway, and its resolution is too low to define the contribution of the two components of the

organelles participating in the intracellular transport: the limiting membrane and the segregated content. Only biochemical analysis of isolated cell fractions can provide these data. Up to the present time, however, only one system, the exocrine pancreas, has been investigated using this approach (25), and even in this system our information is not complete because the characterization of sulfated macromolecules was not detailed and because the subfractionation of zymogen granules did not yield a definite answer as to the membrane or matrix localization of the transported complex carbohydrates.

The system that we used (bovine pituitary slices pulse labeled *in vitro* with both L-[³H]leucine and [³⁵S]sulfate) was selected for two reasons. We knew from the data reported in the accompanying paper that PRL granules can be isolated in purified form from the bovine gland, that MLG can be obtained from these granules after detergent solubilization of the membranes, and that various sulfated macromolecules are components of the PRL granule matrix. Moreover, because of the large size of the gland and its concentration of mammoth cells rich in PRL granules (6), the quantities of the various complex carbohydrates present in purified MLG were sufficient to study their intracellular transport. However, it should be acknowledged that our system has a major limitation, namely the cellular heterogeneity of the pituitary tissue. Thus, comparisons are being made between fractions obtained at two different cellular levels, because MLG originate from the mammoth cells only, whereas the microsomes and the material released into the medium arise from the whole pituitary tissue. This problem cannot be resolved at the present time because the preparations of isolated mammoth cells described in the literature are not pure but are enriched at the most approximately twofold with respect to the intact gland (13).

The results of our cell fractionation experiments are generally in good agreement with the conclusions from the radioautographic studies mentioned above (11, 26). We found that after 15 min of labeling most of the sulfate radioactivity was recovered in the microsomes, which is the cell fraction where Golgi elements are known to be present, while PRL granules were relatively much less labeled. During the first 40 min of chase incubation the radioactivity decreased markedly in microsomes and increased in granules. The observation that the kinetics of sulfate labeling are distinctly different in microsomes and MLG strongly supports the conclusion (arrived at in the preceding paper) that the bulk of macromolecular carbohydrates found in the MLG fraction are not contributed by microsomal contamination but are actual components of PRL granules.

No significant differences were found in the intracellular transport of the various sulfated macromolecules present in the PRL granule matrix. Thus, at all time-points the nondialyzable ³⁵S radioactivity recovered in the MLG fraction could be accounted for in large part (~60%) by sulfated glycoproteins, while in the case of glycosaminoglycans the contribution of heparan sulfate exceeded that of chondroitin sulfate. These data indicate that although the various sulfated complex carbohydrates have distinct molecular and physicochemical features, their assembly into and their disposal from PRL granules may occur by the same processes. In contrast, there was a clear difference between ³H-labeled PRL and ³⁵S macromolecules, because the latter were transported from microsomes to granules in parallel with the hormone but 30–40 min earlier. These results are probably because of the different sites of incorporation of the precursors used to label the two types of molecules.

As previously mentioned, radioautography indicates that [³⁵S]sulfate is incorporated in the Golgi complex (1–3, 25, 26, 30), which is the compartment immediately preceding the granules. This explains why the transport of sulfate-labeled macromolecules to granules occurs relatively early. In contrast, as incorporation of L-[³H]leucine into PRL takes place in the rough-surfaced ER, radioactive hormone molecules spend relatively more time travelling through the Golgi complex before appearing in the granule matrix.

Other interesting findings emerged from the study of ³⁵S macromolecule release from the slices to the extracellular medium. We found that the ³⁵S macromolecules released from the slices during chase incubation far exceeded those lost by the isolated MLG, and that a surprisingly large amount of labeled glycosaminoglycans and glycoproteins was discharged into the medium at high rates during the early periods of chase incubation. Moreover, the SDS PAGE of the incubation media revealed that the kinetics of discharge were not the same for all ³⁵S-labeled macromolecules. In fact, for some components the discharge rate was highest at the beginning of the chase incubation and later declined. Other components showed the opposite pattern (i.e., a progressive increase during chase). These observations indicate that the sulfated macromolecules of the first group become fully available for discharge shortly after their biosynthesis. Thus, they cannot originate from the granules of mammoth cells, where the accumulation of ³⁵S macromolecules continues for at least 40 min during the chase.

In the experiments in which PRL release was almost completely inhibited by dopamine, the release of sulfated macromolecules was not significantly affected. There are at least two possible explanations for this finding. One possibility is that the sulfated macromolecules, although present together with the hormone in the segregated matrix of PRL granules, are not completely released during exocytosis. Rather, they might remain attached to the membrane of the discharged granules and eventually either become externally exposed components of the plasmalemma, or be recaptured by the cytoplasm together with the granule membrane, and possibly reused in the assembly of new secretion granules (9). Alternatively, the sulfated macromolecules of the granule matrix might be completely released during exocytosis, but in an amount too low to be detected above the background of similar molecules released from other sites. However, this second explanation appeared unlikely when we calculated the balance between granule losses and medium gains during chase incubation. During the 40- to 240-min chase period, the [³⁵S]heparan sulfate radioactivity which disappeared from PRL granule matrices of the incubated slices was found to account for ~33% of that appearing in the medium. The corresponding values for glycoproteins and chondroitin sulfate are 24 and 16%, respectively (see Results and footnote 3). Particularly in the case of heparan sulfate the possible contribution of PRL granule matrices appears too large to have been entirely overlooked in our dopamine experiments. Therefore, it seems possible that at least part of the sulfated macromolecules present in the granule matrix remains associated with mammoth cells after exocytosis. This apparently anomalous behavior would appear more likely if sulfated macromolecules were facing the surface of the matrix, in close proximity, or even in association, with the inner surface of the membrane. Such a localization could explain why sulfated macromolecules remain associated with the membrane when the matrix is released by exocytosis, and yet remain with the matrix when the membrane is solubilized by detergents.

Some reported data might be consistent with this hypothesis. For example, cytochemically detectable sugars are concentrated in the outer shell of PRL granule cores (23), and recent evidence indicates that most of the sulfated macromolecules are rapidly solubilized from rat MLG incubated at pH 6 and high ionic strength, even though under these conditions PRL remains aggregated in discrete granule matrices (31). Rosenzweig and Farquhar (26) have also recently observed by radioautography that in rat mammothrophs the ^{35}S labeling of the Golgi region persists even after long chase periods, while under similar conditions most of the labeled PRL is rapidly transported to granules and then discharged (11). Although other explanations are still possible, this finding might reflect the association of ^{35}S macromolecules with the PRL granule membrane, since upon exocytosis these molecules could remain associated with the cell surface and then be recycled to the Golgi complex by endocytosis and vesicular transport (9).

A localization of complex carbohydrates analogous to that which we envisage in PRL granules may also exist in other secretory systems. In the exocrine pancreas, studied by Reggio and Palade (25), the sulfated macromolecules of zymogen granules appear to be more concentrated at the matrix periphery and might be loosely attached to the inner surface of the membrane, because they are increasingly solubilized by repeated washes of membrane pellets. There is also evidence suggesting that in the pancreas sulfated macromolecules may not be released during exocytosis (25), a conclusion which has recently been reported for noradrenergic vesicles (4). In other systems (parotid [2], somatotrophs of the rat adenohypophysis [22], parathyroid gland [20], and hypothalamic-neurohypophysial system [12]), the release of glycosylated macromolecules has been assumed on the basis of the disappearance of radioactivity from the granules, either *in vivo* or during chase incubation. However, because no correlations were made between granule losses and medium gains, it is impossible to establish whether or not the radioactivity left the granules by discharge. Systems in which the release of most granule glycoproteins and glycosaminoglycans was clearly demonstrated are mast cells and adrenal medulla (14, 17). However, it should be noted that the amounts of glycosaminoglycans present in chromaffin and mast cell granules are much larger than those found in the other granules so far investigated, and that in chromaffin granules two glycoproteins, chromogranin A and dopamine β -hydroxylase are the major macromolecular secretion products. Other glycosaminoglycans and glycoproteins are recovered with the isolated granule membranes, where they could be oriented as hypothesized for PRL and zymogen granules (this study and reference 25).

Sulfated glycosaminoglycans and glycoproteins in PRL granules may be involved in the process by which soluble prolactin in the endoplasmic reticulum cisternae is converted to an insoluble form in the granule matrix. Such a possibility is suggested by the synthesis of these complex carbohydrates in the Golgi complex, where granule formation also occurs (19). Reconstitution experiments using pancreatic zymogens (24) suggest that granule membranes, as well as purified glycosaminoglycans, can bind pancreatic secretion products quite efficiently. However, it is clear that in PRL granules the sulfated macromolecules, because of their low concentration (see also reference 32) and probably heterogeneous distribution, cannot by themselves be responsible for hormone insolubilization although they may promote or initiate the process, which could then proceed by other mechanisms such as

interactions between hormone molecules (either directly or mediated by divalent cations). If sulfated glycoconjugates are indeed bound to the inner surface of the granule membrane they could be involved in membrane phenomena, such as those underlying the membrane circulation processes occurring both in the Golgi complex and at the cell surface (see reference 5). At the present time, however, this possibility should be regarded as entirely speculative on account of the lack of specific information on the mechanisms regulating the dynamics of cytoplasmic membranes in secretory cells.

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