Addition of 20 mM ammonium chloride during in vitro chase incubation of [3S]methionine pulse-labeled parotid tissue does not perturb the magnitude or radiolabeling of secretion stimulated by isoproterenol. An apparent inhibition of stimulated output of radioactive secretory granules was observed when ammonium chloride was added immediately postpulse (but not at later time points prior to stimulation) could be accounted for by slowdown in Golgi transit of exocrine secretory protein at a stage prior to completion of terminal glycosylation. Thus, ammonium chloride does not block entry of newly synthesized secretory proteins into the secretagogue-regulated storage granule compartment. By contrast, ammonium chloride increases the output and substantially alters the relative composition of newly synthesized protein in unstimulated secretion. The latter effects could be assigned to stages of intracellular transport that normally occur at chase times >60 min postpulse and thus are focused within the maturing acinar storage granule. Notably, the compositional alterations cannot reflect the preferential exocytosis of immature granules. Taken together, these results suggest that the sorting of exocrine secretory proteins into the secretagogue-regulated pathway may not involve positive selection by a pH-based process initiated in a pregranule compartment. Rather, unstimulated secretion may arise by a negative sorting (or exclusion) process that occurs during compaction of proteins for storage within maturing granules and that is perturbed by weak base addition. Sorted (or excluded) proteins would appear to follow the vesicular (nongranular) secretory pathway that originates in immature granules (von Zastrow, M., and Castle, J. D. (1987) J. Cell Biol. 105, 2675-2684).

We have shown recently that exocrine secretory protein sorting among two distinct export pathways occurs from the content of nascent parotid storage granules (1). Most biosynthetically labeled secretory proteins are retained in storage for eventual release by granule exocytosis. However, a minor fraction of the same labeled species, but clearly differing in relative composition from the large cohort undergoing storage, is diverted during granule maturation for early unstimulated discharge by a nongranular pathway.

Membrane-permanent weak bases have been found to interfere with a number of sorting operations that occur in the intracellular transport pathway. They reroute lysosomal hydrolases into constitutive secretion, thereby precluding delivery to lysosomes, in several cell types (2, 3), and they abolish polarized discharge of certain secretory proteins in an epithelial cell line (4). The altered trafficking has been suggested to result from pH and/or osmotic perturbations that occur upon concentration of weak bases in relatively acidic compartments and that affect either the distribution or the efficacy of specific sorting machinery (5-7). Weak base-elicted rerouting into constitutive secretion of endocrine prohormones that are normally destined for processing and storage in secretory granules (8) has been taken as indirect support for the operation of analogous machinery in selective sorting into the regulated secretory pathway from a pregranule compartment (6). However, this view has been challenged by recent studies showing that weak base treatment does not prevent entry of secretory protein into the site of endocrine storage granule formation (9), nor does it alter the composition of unstimulated secretion (10) that largely originates from granules (rather than pregranule compartments) (10, 11).

Since condensing vacuoles and immature granules in parotid acinar cells are known to be more acidic than the nearly neutral mature storage granules (12, 13), we have been interested in whether weak base accumulation would perturb secretory sorting that occurs from the content of maturing granules. We have examined the effects of ammonium chloride on the entry of newly synthesized secretory proteins into the forming storage granule and on the ensuing sorting into the two export pathways. We show that, even though ammonium chloride slows the transport of pulse-labeled proteins, it clearly does not preclude their entry into the isoproterenol-releasable (secretagogue-regulated) pathway as might be expected for a pregranule pH-dependent sorting process. Further, ammonium chloride elevates two unstimulated pathways of secretion: basal-level granule exocytosis and nongranular release. Finally, weak base addition decreases the compositional

1 M. von Zastrow, P. Arvan, and J. D. Castle, unpublished observations.

2 In keeping with earlier observations that two pathways of unstimulated discharge can be distinguished for exocrine secretory proteins (1, 14), we have qualifed all discussion of unstimulated secretion in this manuscript. Thus, basal level granule exocytosis will refer to the unstimulated discharge of secretion granules (including, where considered, the possibility of preferential exocytosis of newly formed granules). A vesicular (nongranular) pathway will refer to the route taken by polypeptides that are diverted from storage, principally out of the maturing storage granule (1). Operationally, the latter route resembles a "constitutive" pathway (15), especially as recently clarified in endocrine cells (9). We denote it as a diversionary pathway for three reasons: 1) to distinguish it from other constitutive pathways.

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tional selectivity of the latter pathway. Because the effects on nongranular secretion are first observed at chase incubation times ≥60 min, we conclude that perturbation of secretory sorting is occurring within the content of maturing granules.

EXPERIMENTAL PROCEDURES

In Vitro Biosynthetic Labeling of Parotid Tissue and Collection of Secretion—Parotid glands from ad libitum fed male Sprague-Dawley rats (125-150 g) were dissected immediately after animal death by cardiac incision under ether anesthesia. Cleaned tissue was cut into 0.5-mm-thick slices with a Stadie-Riggs knife at 4 °C and preincubated at 37 °C for 30-40 min in two changes of methionine-free RPMI 1640 medium (GIBCO). The slices were then pulse-labeled for 5 min at 37 °C in fresh methionine-free medium (8 ml for tissue from six glands) supplemented with 1 or 2 μCi of [35S]methionine (600 Ci/mmole, 1 μCi = 37 GBq; Du Pont-New England Nuclear) and subsequently were washed with four 10-ml changes of warmed and oxygenated chase medium, RPMI containing excess (150 μg/liter) L-methionine, 10 mM Hepes buffer (pH 7.3), 20 mM either sodium chloride (control) or ammonium chloride, and 1 μM concentration of each of the autonomic inhibitors atropine, phentolamine, and propranolol. The tissue was then divided equally among aliquotized 10-ml chase incubation flasks, each containing 1.5 ml of chase medium. At the times indicated, tissue immediately postpulse, and, every 30 min thereafter, the medium in each sample was completely removed for further processing and replaced with fresh medium containing either additional sodium chloride or ammonium chloride, as specified. Collected medium was centrifuged at room temperature for 5 min at 11,000 x g to remove traces of cellular debris and stored as frozen aliquots for further assays. During the final 30-min chase time interval of each experiment, incubation media contained 20 μM isoproterenol in place of autonomic antagonists in order to stimulate large scale exocytosis of storage granules. Upon termination of incubation, the tissue in each sample was washed with 10 ml of medium and homogenized in 1.5 ml of 0.15 M NaCl to provide comparative assay samples.

Digestion of Radiolabeled Tissue Samples with Endoglycosidase H—Parotid tissue slices were pulse-labeled and divided for subsequent chase incubation in either control medium or ammonium chloride medium as described above. At each time point, tissue samples were withdrawn from each medium and homogenized in ice cold water; the homogenates were filtered through 20-μm nylon screen and frozen. For each digestion with endoglycosidase H (endo H; from Genzyme, Boston, MA), thawed samples were denatured by boiling 2 min in 0.1% SDS, diluted to give 5 μg/ml sodium phosphate (pH 6), 0.1% Triton X-100, 0.02% SDS, and a protein concentration of 0.1 mg/ml. Endo H (100 μIU/ml) or buffer (for mock digestion) was added, and the samples were incubated 16-20 h at 37 °C (sufficient for complete digestion of susceptible oligosaccharides on parotid secretory glycoproteins). Digestions were stopped by boiling following addition of SDS-containing electrophoresis sample buffer and were analyzed by SDS-PAGE, fluorography, and densitometry.

Biochemical Analyses—One-dimensional SDS-PAGE (16) of tissue and medium samples containing equal amylase activity was performed in either 12.5% acrylamide gels (1.5 mm thick) or 10-15% acrylamide gradient gels (1 mm thick). Fixed and Coomasie Blue-stained gels were washed and impregnated with safilcyc for fluorography essentially according to Chamberlain (17). Quantitation of radioactivity in individual gel bands was performed by two procedures: 1) integration of densitometric scans of fluorographs exposed in the linear range on Kodak XAR-5 film; 2) excision and digestion of gel bands and quantitation by liquid scintillation spectrometry (1, 14).

α-Amylase was assayed according to Bernfeld (18), lactate dehydrogenase according to Schnaar et al. (19), and protein according to Markwell et al. (20).

RESULTS

Ammonium Chloride Does Not Affect Either Cell Integrity or Secretagogue-regulated Discharge of Stored Secretory Proteins

We studied the effects of ammonium chloride on protein secretion at a concentration of 20 mM, which is within the range used to disrupt protein sorting in other systems (7, 9, 10, 21). Addition of ammonium chloride during in vitro incubation of parotid tissue slices caused no detectable effect on cell integrity, as measured by release of cytosolic lactate dehydrogenase into the incubation medium. In four separate experiments, the release of lactate dehydrogenase was linear with time and did not differ significantly between control and ammonium chloride-treated specimens (1.8 ± 0.31 versus 1.9 ± 0.35% per h, respectively).

Parotid acinar cells are highly specialized for stimulus-regulated secretion as characterized by a massive discharge of stored secretory proteins upon administration of β-adrenergic agonists (22). Ammonium chloride caused no change in this general characteristic. Addition of ammonium chloride at any time during a 3-h incubation period did not diminish detectably the pool of stored amylase that was released upon addition of isoproterenol (Fig. 1a).

Ammonium Chloride Has Little Effect on the Delivery of Newly Synthesized Proteins to Storage Granules That Undergo Secretagogue-regulated Exocytosis

Most secretory proteins in parotid acinar cells are exported by stimulus-regulated exocytosis after extended storage in secretory granules (1). Since weak base treatment has been claimed to divert endocrine proteins from entry into secretion granules (8), we investigated the effect of ammonium chloride on entry of newly synthesized parotid proteins into the isoproterenol-regulated storage pool. We pulse-labeled parotid tissue in vitro, added ammonium chloride to the incubation medium at various chase times, and assayed delivery by measuring discharge of radiolabeled secretory proteins in response to the β-adrenergic stimulus at the 3-h chase. Under normal conditions, essentially all secretory proteins are contained in relatively mature granules at this time, and there is, as yet, little unstimulated exocytosis of labeled granules (1). Ammonium chloride slowed the appearance of radiolabeled proteins in stimulated secretion only when added immediately post-pulse; no inhibition of transport to the regulated storage compartment was detected when weak base was added at ≥30 min (Fig. 1b). We were unable to identify any differential effects on transport of individual secretory species; in the presence of ammonium chloride, all granule content proteins were transported with equal efficiency to the storage compartment. This was true even when the weak base was added immediately postpulse (Fig. 1c).

In order to determine the site where ammonium chloride retards intracellular transport, we examined the kinetics of processing of N-linked oligosaccharides of parotid glycoproteins following immediate postpulse addition of weak base. As shown in Fig. 2a, ammonium chloride does not alter detectably the onset of resistance to endoglycosidase H digestion (in agreement with the results of others (e.g. Refs. 21 and 23)) for the two glycoproteins, M, = 36,000 and 22,000 (p36 and p22, identified in Fig. 1c). By contrast, completion of terminal glycosylation, which can be visualized only for p22 as an increase in apparent Mr, is substantially delayed (Fig. 2b). Thus, we attribute the partial inhibition of stimulated discharge of radiolabeled proteins caused by early am-
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Ammonium chloride addition on isoproterenol-stimulated secretion of amylase activity and radiolabeled proteins. Parotid tissue slices were pulse-labeled (5 min) with [35S]methionine and chase-incubated for 3 h in medium containing autonomic inhibitors (1 μM concentration each of atropine, phentolamine, and propranolol) and then 30 min in the presence of 20 μM isoproterenol (no inhibitors present). Complete medium changes were made at 30-min intervals and allowed for replacement of a 20 mM NaCl supplement (control medium) with a 20 mM ammonium chloride medium. Ammonium chloride was added to separate samples for the duration of the experiment at 0, 30, 60, 90, and 120 min (B–F, respectively in panel a) but not to a control (A in panel a). At the end of the experiment, each tissue sample was homogenized and assayed for amylase in parallel with medium samples. a, percent release of amylase ([(medium/medium + tissue) × 100], showing that ammonium chloride does not detectably alter the magnitude of secretagogue-regulated exocytosis. b, isoproterenol-induced discharge of radiolabeled secretory proteins (quantitated by summing the radioactivity in dissolved gel slices from all major secretory polypeptides). Data are expressed relative to the control (100%) and show that stimulated output is diminished only when ammonium chloride is added immediately postpulse. c, sample fluorographs of isoproterenol-induced secretion showing that its relative radiochemical composition following ammonium chloride addition at various chase times prior to stimulation does not differ from that of the control (C). Samples subjected to SDS-PAGE contained equal amylase enzyme activity, and the decreased specific radioactivity with ammonium chloride addition immediately postpulse (0 min) is evident.

Corresponding calculations based on the endo H-induced change in integrated density of the M9 = 16,000 band agreed closely with those presented for M9 = 20,000 + 22,000, O control medium; ammonium chloride medium. The corresponding time courses for endo H digestion of a M9 = 36,000 parotid glycoprotein following incubation in the absence and presence of ammonium chloride indicate a very similar lack of effect of weak base on early oligosaccharide processing (not shown). b, time course of recovery of the M9 = 20,000 precursor glycoprotein to its M9 = 22,000 product in the presence and absence of ammonium chloride. The amounts of M9 = 20,000 (O); and 22,000 (△△) polypeptides have been normalized to the same total M9 = 20,000 + 22,000 integrated density for all samples. O△△, control medium; △△, ammonium chloride medium. The inset illustrates the relative amounts of M9 = 20,000 and 22,000 polypeptides after a 20-min incubation in the absence or presence of ammonium chloride (AC). The weak base-induced delay in processing is evident; quantifying processing to terminal glycosylation is based on a separate demonstration that the M9 = 22,000 species, but not the M9 = 20,000 precursor, is galactosylated (not shown).

Proteins packaged in granules was affected similarly; no change was noted in the relative amounts of individual secretory proteins exported (according to comparative SDS-PAGE analysis), and this composition reflected closely that packaged in granule content. Following removal of ammonium chloride from the incubation medium, this nonelective increase in secretion was reversed rapidly and completely, with a t1/2 of about 30 min (Fig. 3b). In order to discern whether the immediate increase in total output reflects discharge of older (previously stored) or newly synthesized proteins, we subjected parotid preparations to pulse-chase incubation and examined the specific radioactivity of amylase secreted within the first 30 min after ammonium chloride addition (when the full effect is realized) relative to a control for the same interval. In all cases, there was no difference in the specific radioactivities of control and treated samples until after 30 min (broken line in Fig. 3d). Therefore, the immediate effect of ammonium chloride is not on the output of newly synthesized proteins. Rather, it probably reflects slight elevation in basal level exocytosis of stored granules.

Increased Output of Recently Synthesized Proteins During Granule Maturation—In order to evaluate the effects of ammonium chloride on the output of recently synthesized proteins from parotid, we examined the unstimulated secretion of biosynthetically labeled proteins in pulse-chase experi-
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FIG. 3. Ammonium chloride effects on unstimulated secretion of amylase and radiolabeled secretory proteins. a, output of amylase activity. The same experimental protocol was used as in Fig. 1, with ammonium chloride being added to separate samples at successive 30-min time intervals up to 120 min with continued incubation until 180 min. The data from the separate samples representing different times of ammonium chloride addition have been pooled according to the same length of exposure to the weak base and expressed relative to output from a control sample (no ammonium chloride) over the same interval. The small (1.6- to 2-fold), rapid increase in amylase secretion. Separate tissue samples were incubated with ammonium chloride-containing medium were transferred to control (NaCl-supplemented) medium at 60 and 120 min, respectively, and incubated until 210 min. The change in output rate upon weak base removal has been compared in the two samples according to an equal time interval after addition of control medium and has been expressed relative to output from a control sample that was never exposed to ammonium chloride. The rapid (t½ = 30 min) and complete reversibility in elevated output can be seen. b, unstimulated secretion of [35S]methionine pulse-labeled secretory proteins following ammonium chloride addition at successive 30-min intervals from 30 to 120 min and maintained for the remaining incubation were used to quantitate the total released radioactivity at each 30-min time interval, aliquots of incubation media were subjected to SDS-PAGE and fluorography, and the labeled bands corresponding to all major secretory proteins in each sample were excised, digested, and counted. Total radioactivity is the sum of these counts corrected to the total incubation volume. The results show clearly that elevated secretion is detected only after a 60-min chase (even with earlier weak base addition) and can be elicited at a comparable rate after a 120-min chase. d, increase in total radiolabeled protein secretion and amylase specific radioactivity after ammonium chloride addition. The data are recalculated from the experiment shown in panel c after pooling according to equal time of exposure to weak base, irrespective of the time of ammonium chloride addition. Values are expressed relative to the appropriate interval for the control specimen. Amylase specific radioactivity was determined from the total radioactivity present in the αα, = 58,000 amylase band (e.g. band A, Fig. 4a) for samples of equal amylase enzyme activity. In panels a, b, and d the vertical lines show the range of the data when values from separate samples were pooled according to a common time interval after addition (a, d) or removing (b) ammonium chloride. The graphs are drawn through the means.

FIG. 4. Effect of ammonium chloride on the radiochemical composition of unstimulated secretion determined from [35S] methionine-labeled samples from pulse-chase incubation. Incubation medium was entirely replaced at 30-min intervals during the 180-min chase, and samples were taken for analysis of secretory composition. A 30-min incubation with 20 μM isoproterenol was performed at 180–210 min to enable direct comparison of unstimulated and stimulated secretion. a, fluorographs of the entire incubation sequences for a control (upper panel) and a sample in which ammonium chloride medium was added at 60 min (lower panel). 2.0 units of amylase activity was loaded in each lane for SDS-PAGE. The diminished compositional selectivity of unstimulated secretion following weak base addition, particularly the increased relative incidence of p32 and p25, is evident. The results shown are representative of three experiments. b, quantitation of the ammonium chloride induced change in radiolabeled discharge (relative to a control sample) for individual secretory polypeptides p22, p25, and p32. Fluorographs from samples where ammonium chloride was added at 60, 90, and 120 min and maintained for the remaining incubation were used along with the control. Data from the different samples were grouped together according to the same length of exposure to ammonium chloride (irrespective of the time of weak base addition) and were expressed as a ratio using the control sample for the corresponding interval. As in Fig. 3, the vertical lines show the range of the data at each interval when pooled this way.

(1). In contrast to the immediate effect of ammonium chloride on the output of previously stored proteins described above, the effect on recently synthesized proteins always was delayed in onset, occurring with a t½ between 30 and 60 min (Fig. 3d). Despite its slower onset, the increase in the output of recently synthesized proteins (about 4-fold) exceeded the increase in output of previously stored proteins (about 2-fold; compare d and a of Fig. 3). Another distinguishing characteristic is that, in contrast to the rapid reversal of the ammonium chloride-increased output of previously stored proteins, the reversal of increased output of recently synthesized proteins was delayed and only partial; 50% reversal occurred approximately 120 min after removal of weak base (not shown).
Examination of reversal at later times was obscured by the onset of basal level exocytosis of labeled granules.

**Diminished Compositional Selectivity of Unstimulated Secretion Reflects the Nongranular (Diversionary) Pathway**—Unstimulated secretion of recently synthesized proteins by vesicular divulsion from maturing parotid granules, under normal conditions, is compositionally selective. The p22 glycoprotein is substantially over-represented whereas 25- and 32-kDa (p25 and p32) polypeptides are practically negligible in relation to their relative proportions in storage granules of all stages of maturity (1). Ammonium chloride reduced substantially the compositional selectivity of this secretion; relative output of p25 and p32 rose to the greatest extent while relative output of p22 rose only slightly. This differential behavior is evident merely by inspection of fluorographs of the radiolabeled secretion resolved by SDS-PAGE (Fig. 4a) and is detailed more explicitly by quantitative comparison among the polypeptides discussed (Fig. 4b). The changes in the output of radiolabeled proteins did not occur in proportion to their relative amounts in secretory granules as would be expected if an increase in exocytosis of labeled granules were responsible for the effect. Thus, for example, the ratio of labeled p25/p32 in the granules (closely corresponding to the ratio in unstimulated tissue and in stimulated secretion) is 1.1, yet the relative effect of weak base on p25 is twice that on p32 (Fig. 4b). As well, the amount of labeled p25 released remained over-represented in relation to granule levels.

The time course of changes in compositional selectivity paralleled the time courses of increased total output of recently synthesized proteins and increased amylase specific radioactivity (Fig. 3d) and thus was distinguishable from the time course of increased basal level granule exocytosis (Fig. 3a). Finally, the ammonium chloride diminution of compositional selectivity was reversed only partially at 120 min after removal of weak base (not shown).

**DISCUSSION**

This series of experiments shows that a rather high concentration of ammonium chloride does not affect parotid acinar cell viability or stimulus-regulated secretion, but does exert multiple, experimentally distinguishable effects on the trafficking and unstimulated release of exocrine proteins. Ammonium chloride slowed intracellular transport only when added immediately postpulse; no inhibition of transport was detected when addition was made just before or during passage of pulse-labeled proteins into granules, i.e. at ≥30 min (Fig. 1b and Ref. 1). The inhibition could be accounted for by slowdown in Golgi transit prior to completion of terminal glycosylation (Fig. 2). Thus, the weak base does not block entry of exocrine proteins into forming granules. This finding is in discord with the proposal that pH-dependent sorting (perturbable by acidotropic agents) is the general mechanism by which secretory proteins enter the storage granule. The relatively mild acidity (12, 13) and substantial buffering capacity of the granule interior (13) may diminish the effectiveness of acidotropic agents in the parotid system. However, it is difficult to rationalize that these properties would obscure pH-dependent sorting in view of the substantial perturbations of secretory traffic induced by ammonium chloride at later stages of granule maturation (where the acidity is lower and the buffering capacity may be greater due to the increased concentration of packaged proteins). In agreement with the present results, Mains and May (10) have shown that weak bases do not inhibit entry of hormone precursors into storage granules in pituitary AtT20 cells under conditions where cell viability was ensured (5 mM ammonium chloride + 5 mM methylamine or ≤40 μM chloroquine). Higher levels of chloroquine (200 μM) were shown to be cytotoxic (10) and may have created a misconception in an earlier study where chloroquine was reported to block the same process in AtT20 cells (8). One may suppose from our data and that of Mains and May (10) that selective targeting to the nascent granule may not be involved in secretory sorting, or even that packaging in nascent granules may represent a “default” route for proteins that are not directed elsewhere. However, we do not feel that the information at hand is sufficient to support such conclusions. Particularly in view of the report that monensin blocks the packaging of zymogens in exocrine pancreas (15), we continue to regard pH (or other ion)-dependent targeting as a possible factor in the formation of secretory granules.

In contrast to its minimal influence at pregranular sites of sorting, ammonium chloride perturbed substantially the sorting and unstimulated secretion of biosynthetically labeled proteins after their arrival in nascent granules. Two separate effects on unstimulated secretion of older and recently synthesized proteins, respectively, were distinguished according to kinetics of onset and reversal. The effect on secretion of older proteins was a nonselective increase in output that is probably mediated by a moderate increment in the rate of basal level exocytosis of mature granules. Such exocytosis is the major source of unstimulated secretion in untreated exocrine cells (1, 14), and this continues to be true in the presence of ammonium chloride. The effect on secretion of recently synthesized proteins was more pronounced and involved both an increase in output of proteins from the content of maturing granules and a large decrease in its compositional selectivity. Significantly, some compositional selectivity was still observed in the presence of weak base, and it could be used to rule out the possibility that exocytosis of immature granules is the source of increased output of radiolabeled proteins. Thus, we conclude that ammonium chloride both increases the volume of secretory traffic through the nongranular, diversionary route and decreases (but does not abolish) the selectivity of granule-based sorting into this pathway. As noted previously (1), there may be bona fide differences between these exocrine cells and certain endocrine cell types in which preferential exocytosis of young granules is thought to occur (10, 11). However, we are struck by the apparent analogy of our findings to the occurrence of diversion of a nongranular secretory pathway and compaction of stored endocrine proteins at a common site as elegantly illustrated by Tooze et al. (9). Thus, we feel that there may be some merit to exploring further whether vesicle-mediated diversion of secretory products is sustained during endocrine storage granule maturation as we are suggesting for exocrine granule maturation.

Two ways come to mind as to how granule-based sorting could occur to account for the present results. First, secretory proteins could be sorted selectively into the diversionary, nongranular pathway by “positive selection” through interaction with the membrane or even a receptor-mediated process (analogous diversion of lysosomal hydrolases from a post-Golgi site has been implicated in other cell types (24)). Second, proteins undergoing storage could be sorted by selective retention in maturing granules, reflecting interaction between polypeptides undergoing condensation and osmotic stabilization (25-27). The efficiency of content interactions during endocrine granule formation, and their sensitivity to ammonium chloride addition, have been emphasized clearly (9). In this case, the diversionary export pathway would carry primarily “negatively sorted” species, reflecting lower tendency to be retained in content aggregates. We favor the second possibility as supported by the present data: ammonium chlo-
ride not only affects the selectivity of diversionary export (compatible with either possibility), but also increases the overall amount of secretory proteins diverted (suggesting the latter process, as the former predicts an overall decrease in amount). Thus, the weak base may alter diversionary export by decreasing the stability of interactions between packaged proteins. Whether this occurs by perturbation of intragranular pH per se remains to be determined.

In conclusion, the present results corroborate and extend our earlier deduction that secretory sorting in parotid acinar cell includes granule-based diversion into an alternate export route. Although our data do not support the earlier proposal of pH-dependent sorting among secretory pathways from a pregranule site, we continue to consider the possible role of pregranular selection in secretory sorting. Pregranular and granule-based sorting operations are not mutually exclusive, and the conduct of secretory traffic in complex regulated cell types may require multiple sites of molecular sorting. This suggestion may be particularly applicable to exocrine cells where secretory storage is a major activity and where constitutive pathways to separate cell surfaces are present. Further exploration of these possibilities will require the use of more selective experimental tools than membrane-permanent weak bases.

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