Quantitative Subcellular Study of Apical Pole Membranes from Chicken Oxyntic Cells in Resting and HCl Secretory State

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Abstract. Vertebrate oxyntic cells, responsible for gastric HCl production, undergo a remarkable morphological reorganization in relation to their secretory cycle. In resting state, the luminal surface of the cells is smooth; a peculiar system of endocellular membranes, the tubular system, occupies the luminal cytoplasm. Actin filaments frame a cortical network between the tubular system and the luminal plasma membrane. With the onset of HCl secretion, the tubular system becomes incorporated into the luminal plasma membrane. Villous processes containing microfilaments fill the secretory surface. This morphological reorganization of membranes and cytoskeletal matrix could regulate HCl secretion by translocation of membranes containing the proton pump from the endocellular compartment to the secretory surface.

In this paper, we describe the isolation of membranes that selectively belong to the tubular system or to the cytoplasmic processes of the secretory surface of chicken oxyntic cells. Chicken oxyntic cells are the main cellular component of the proventricular glands. A resting state was obtained after cimetidine treatment, whereas the HCl-secretory state was induced by histamine.

We present a comparative analysis of resting and stimulated chicken gastric glands by quantitative subcellular fractionation. The HCl secretory state was related to specific modifications in membrane fractions derived from the secretory pole of oxyntic cells. Morphological and functional reorganization of oxyntic cells was closely correlated with changes in: the sedimentation pattern of the marker enzyme of the apical pole membrane (K-NPPase), the total activity of K-NPPase and nonmitochondrial Mg-ATPase, the valinomycin dependence of K-ATPase, and polypeptides that cosediment in purified membrane fractions.

Changes in the distribution pattern of K-NPPase after fractionation of histamine-stimulated glands were consistent with the replacement of the small vesicles typical of resting glands by dense membrane profiles, analogous to the luminal processes of stimulated oxyntic cells. SDS-PAGE showed that, in purified membrane fractions of stimulated glands, the concentration of 28-, 43-, and 200-kD polypeptides increased while that of 95- and 250-kD polypeptides decreased.

The present results define the tubular system of oxyntic cells as an organelle with properties different from those of endoplasmic reticulum, mitochondria, and plasma membrane. The biochemical and physicochemical properties of this membraneous system changed when the organization of the membranes and the cytoskeletal matrix of the apical pole was modified by the onset of HCl secretion.

It is widely accepted that translocation of organelles, local movements of the plasma membrane, and changes in cell shape depend on the integrated action of cellular membranes and the cytoskeletal network (Jacobson, 1983; Geiger, 1983). The existence of different types of microfilament-membrane interactions, probably related to specific cellular functions, has been clearly established (Tilney, 1983; Bennett and Condeelis, 1984; Jung et al., 1984, and Mooseker et al., 1984). Transient microfilament-membrane interactions that occur in epithelial cells during cyclic secretory processes are less documented (Vial and Garrido, 1976; Tramontano et al., 1982; Osawa et al., 1984).

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Vertebrate oxyntic cells provide an excellent model for studying microfilament-membrane interactions during transient events. The membranous systems proper of the secretory pole, the tubular system, and the plasma membrane change their relationship with the actin cytoskeleton during the secretory cycle of HCl (Vial and Garrido, 1976; Vial et al., 1979, 1985). In the resting state, the luminal surface of oxyntic cells is smooth, and an extensively developed tubular system occupies the apical cytoplasm. Actin filaments comprise a cortical network between the plasma membrane and the tubular system (Koenig et al., 1981*a*). In cells actively secreting HCl, the tubular system diminishes to point of disappearance. At the same time the secretory surface markedly increases and becomes filled with cytoplasmic processes

which expand the apical membrane area ~10-fold (Helander et al., 1972). These processes contain parallel arrays of actin microfilaments connected to the plasma membrane by regularly spaced bridges (Vial and Garrido, 1976; Vial, 1982; Vial et al., 1985).

Thus, stimulation of HCl secretion induces the reorganization of both the actin cortical network and the membranous systems of the secretory pole (Vial et al., 1985). While the membranes of the tubular system become associated with microfilaments, molecular interactions between actin and other cytoskeletal proteins of the cortical network change, resulting in the reorganization of the secretory pole membranes (González et al., 1981; Black et al., 1982; Vial et al., 1985; Dabiké et al., 1986). This structural reorganization precedes the onset of HCl secretion (Jiron et al., 1984). Upon cessation of stimulus, the tubular system is reformed. These morphological changes could reflect the regulation of the output of gastric HCl by translocation of the membranous system containing the K/H-ATPase from the endocellular compartment to the luminal surface of the cell (Smolka et al., 1983). K/H-ATPase, recognized as the gastric proton pump (Forte et al., 1980; Sachs et al., 1982; Wallmark et al., 1983), seems to be involved in the terminal steps of the acid secretory process (Fellenius et al., 1981). The catalytic cycle of gastric K/H-ATPase includes a K^+-stimulated phosphoprotein phosphatase, insensitive to ouabain, which has often been studied as a K^+-*p*-nitrophenylphosphatase (K-NPPase)[1] (Wallmark et al., 1983; Jackson et al., 1983; Hirst and Forte, 1985). Cytochemical studies have shown that, in oxyntic cells, the subcellular localization of the K-NPPase drastically changes with the onset of acid secretion. In resting cells, the K-NPPase reaction product is preferentially associated with the membranes of the tubular system, while in secreting cells it is concentrated in the plasma membrane of the elaborated secretory surface (Koenig, 1984; Fujimoto et al., 1986).

HCl physiological secretagogues, acting at the basolateral plasma membrane, are known to activate the acid pump through second messengers (Berglindh, 1984). Although it has been found that in vivo stimulation of HCl secretion induces changes in the properties of membranes containing K/H-ATPase (Wolosin and Forte, 1981*a*, *b*, 1983; Im et al., 1984) the molecular mechanism regulating the activation of HCl secretion is not yet fully understood.

The differences in structural organization of resting and secreting cells may be used to isolate membrane fractions corresponding either to the tubular system or to the secretory surface cytoplasmic processes. Chicken gastric glands are a suitable starting material for the preparation of oxyntic cells. Although these cells secrete both acid and pepsinogen, chicken stomachs release more H^+ per kilogram of body weight than any mammal so far examined (Long, 1967; Burhol, 1982). Oxynticopeptic cells account for over 80% of the cellular mass in glandular lobules (Farner, 1960). The similar reorganization of oxyntic cells from a number of vertebrates, including chickens, has been well documented (Vial, 1982; Vial et al., 1979; Forte et al., 1981*a*). In chickens, the morphofunctional state of oxyntic cells can be regulated in vivo to obtain a defined resting or HCl secretory state (Toner, 1963; Koenig, 1984).

This paper presents a quantitative biochemical analysis of chicken gastric glands in two secretory states: active HCl secretion induced by histamine and a resting state after cimetidine administration. Morphological and functional changes of oxyntic cells were closely correlated with modifications in the sedimentation pattern of the apical pole membranes. Differences were found in the total activity of K-NPPase and Mg-ATPase associated with these membranes, as well as differences in the polypeptides that cosedimented in the purified membrane fractions.

Materials and Methods

Animals and Drug Administration

White Leghorn chickens (*Gallus domesticus*) weighing ~1.5 kg were used. They were deprived of food 24 h before the experiment with water ad libitum.

To obtain resting glands, two doses of cimetidine were injected intraperitoneally (2 mg/kg body weight) 15 and 1 h before the experiment. To stimulate HCl secretion, 0.7 mg of histamine base per kg of body weight were injected subcutaneously 40 min before the birds were killed. The doses of cimetidine and histamine were chosen on the basis of studies correlating the secretory response and the morphological organization of the oxyntic cell's apical poles in fasted chickens.

Preparation of Homogenates

Glandular stomachs were quickly removed from birds killed by decapitation, and the pH of the gastric fluid was measured with pH paper to test HCl secretion. The opened proventriculus was left in a petri dish on ice, and the glandular lobules were surgically dissected from the mucosa and the muscle layers. Gastric glands were filtered through a tissue press (model 141; Harvard Apparatus Co. Inc., South Natick, MA) and weighed. Usually, 1–2 g of gland were obtained from each stomach. The tissue was placed in a Teflon/glass grinder model A (Thomas Scientific, Philadelphia, PA) containing 5 ml of ice-cold homogenization medium (buffered sucrose: 0.25 M sucrose, 3 mM imidazole-HCl, pH 7.4) per g of tissue wet weight. Homogenization was performed with two strokes of the pestle driven at 1,000 rpm at 0°C.

Subcellular Fractionation

Homogenates were fractionated by differential centrifugation as described by de Duve et al. (1955) for rat liver, except that the nuclear (N) and heavy mitochondrial (M) fractions were sedimented together (NM fraction). The light mitochondrial (L), microsomal (P), and final supernatant (S) fractions were obtained from the NM supernatants, essentially as described by these authors.

In other experiments, the NM supernatants were fractionated by filtration on Sepharose 2B. A total particulate fraction, corresponding to the sum of the L and P fractions (LP), and a soluble fraction (S), containing the soluble components of the homogenate (Bronfman and Beaufay, 1973), were obtained by this method.

The LP fraction was further subfractionated by isopycnic equilibration in metrizamide-sucrose linear density gradients, extending from 1.05 to 1.22 g/ml. The gradients (5 ml) were prepared with a density-gradient former (Beckman Instruments, Inc., Palo Alto, CA). The LP fraction (prepared in buffered sucrose) was used for the light solution and a 39% wt/vol solution of metrizamide in buffered sucrose was used for the heavy solution. The gradients were centrifugated for 60 min at 39,000 rpm in a SW-65 rotor (Beckman Instruments, Inc.) at 6–10°C. After centrifugation, 12–13 fractions were collected by slicing the tube with a tube slicer (Beckman Instruments, Inc.) fitted with a cooling jacket, at 0–2°C. Density measurements in each fraction were performed by the procedure of Beaufay et al. (1964).

Results from subcellular fractionation experiments are presented in the form of normalized histograms constructed according to procedures previously described (de Duve et al., 1955; Beaufay et al., 1964; Leighton et al., 1968).

1. *Abbreviations used in this paper*: K-NPPase, K^+-*p*-nitrophenylphosphatase; L, light mitochondrial fraction; M, heavy mitochondrial fraction; N, nuclear mitochondrial fraction; P, microsomal fraction; S, supernatant.

Enzyme Assays

Established procedures were used for the determination of marker enzymes: catalase for peroxisomes (Leighton et al., 1968), NADPH cytochrome *c* reductase for microsomal vesicles (Beaufay et al., 1974), cytochrome *c* oxidase for mitochondria (Cooperstein and Lazarow, 1951), and acid phosphatase for lysosomes (Appelmans et al., 1955). Protein was measured by the method of Lowry et al. (1951), as modified by Wattiaux et al. (1978), using BSA as standard. Phosphoglucomutase, a soluble-compartment marker, was determined by the method of Ray and Roscelli (1964), as described by Bronfman et al. (1984). Assay of 5'-nucleotidase was performed according to Ray and Forte (1974). (K-EDTA)-ATPase, a marker of myosin (Ostlund et al., 1978), was quantified as described by Koenig et al. (1981*a*).

ATPase activities were assayed as reported for gastric glands (Ganser and Forte, 1973*a*, *b*; Forte et al., 1975; Saccomanni et al., 1977) with minor modifications. The reaction mixture contained 2 mM $MgCl_2$, 2 mM ATP, 0.1 mM ouabain, and 10 mM PIPES-NaOH buffer, pH 6.8, in a final volume of 1.0 ml. Incubations were performed at 37°C. Activation by K^+ was determined in the presence of increasing KCl concentrations (from 0.1 up to 50 mM). The amount of valinomycin necessary to reach a final concentration of 10 μM was added to the incubation medium. ATPase activities were defined after Lee et al. (1979): Mg-ATPase is the activity detected in the absence of K^+; K-ATPase is the increase in activity of Mg-ATPase after addition of K^+; valinomycin-stimulated K-ATPase is the additional increase in activity of K-ATPase in the presence of the ionophore. K-NPPase was assayed as for ATPases, except that 25 mM Tris-HCl, pH 7.2, was used as buffer, and 5 mM *p*-nitrophenylphosphate as substrate (Forte et al., 1975). K-NPPase is defined as the increase in activity induced by 20 mM KCl in the basal activity detected in the presence of Mg^{++}. The kinetics of all enzymes were examined in detail to find the optimum conditions of the assays and, in particular, to determine the range within which the measured velocities were proportional to enzyme concentration. Enzymatic assays were performed in aliquots of subcellular fractions suitably diluted. Protein concentrations of aliquots added to the assay medium were: 10–300 μg for catalase, 15–150 μg for NADPH-cytochrome *c* reductase, 0.5–5 μg for cytochrome oxidase, 50–500 μg for acid phosphatase, 10–150 μg for phosphoglucomutase, 50–500 μg for 5'-nucleotidase, 500 μg for (K-EDTA)-ATPase; 1–50 μg for ATPases, and 5–200 μg for K-NPPase.

Units of enzyme activity were defined as the amount of enzyme causing the transformation of 1 μmol of substrate per min in the conditions of the assay. For catalase, 1 U of activity is the amount of enzyme destroying 90% of the substrate in 1 min in a 50-ml vol (Leighton et al., 1968). 1 U of cytochrome *c* oxidase corresponds to the amount of enzyme that causes the decadic logarithm of reduced cytochrome *c* to decrease by 1 U/min per 100 ml incubation mixture (de Duve et al., 1955).

Substrates and enzymes were obtained from Sigma Chemical Co. (St. Louis, MO). Metrizamide (2-[3-acetamido-5-*N*-methylacetamido-2-4-6-triiodobenzamido]2-deoxy-D-glucose) was obtained from Nyegaard and Co. (Oslo, Norway).

Morphological Examination

Lobules from all gastric glands were fixed in 3% glutaraldehyde in PES (100 mM PIPES, pH 6.9, 100 mM KCl, 2 mM $MgCl_2$, and 0.25 M sucrose) for 6 h at room temperature. Fixation was performed immediately after extraction of the proventriculus and within 15 min after dissection at 4°C. Glands were washed overnight at 2°C in PES and postfixed for 1 h in 1% OsO_4 in 0.1 M cacodylate buffer, pH 7.2. The tissue was stained with aqueous 1% uranyl acetate, dehydrated in acetone, and embedded in Epon. Small aliquots of selected fractions were poured in an ice-cold solution containing 3% glutaraldehyde in PES. After 2 h the fractions solidified by fixation and were processed as described above. In some experiments, fractions were fixed in glutaraldehyde and tannic acid (Brunschwig et al., 1982). Thin sections were stained with 2% uranyl acetate and lead citrate and examined in a Siemens Elmiskop 102 electron microscope.

Samples of fractions from the metrizamide gradient were negatively stained with 1% uranyl acetate or 0.5% phosphotungstic acid.

SDS-PAGE

SDS-PAGE was performed according to Laemmli (1970). Fractions were dissolved in a solution containing 75 mM Tris-HCl, pH 6.8, 10% glycerol, 5% vol/vol 2-mercaptoethanol, 2% wt/vol SDS, and 0.002% bromophenol blue, and heated for 3 min at 100°C. 3% stacking and 10% separation acrylamide gels were used. Molecular weight markers were filamin (250,000), spectrin (220,000), myosin heavy chain (200,000), clathrin (180,000), α-actinin (100,000), IgM1 (78,000), actin (43,000), DNase (31,000), IgM2 (20,000), and lactoglobulin (18,400). Quantitative scanning densitometry of Coomassie Blue–stained gels was used to determine the relative amount of polypeptides present in each fraction. The gels were scanned at 605 nm in a microdensitometer (KII; Canalco) with a Disc-Integrator and Printer System (model A-25; Varian Associates, Inc., Palo Alto, CA).

Results

Correlation between Structure of Chicken Oxyntic Cells and Gastric HCl Secretory State

The morphological analysis of glands used for fractionation showed a very good correlation between oxyntic cell ultrastructure and gastric HCl secretory state. In each stomach, nearly 100% of the cells examined had the same structure. Fig. 1 typifies the ultrastructure of gastric glands isolated from secreting (Fig. 1, *a* and *b*) and resting (Fig. 1, *c* and *d*) stomachs. After histamine stimulation, chicken oxyntic cells showed a prominent secretory pole surrounded by an intercellular cleft filled with the complex processes of the luminal surface (Fig. 1 *a*). The cytoplasmic matrix inside these processes contained microfilaments whose associations to the plasma membrane were occasionally observed (Fig. 1 *b*). In resting state, after fasting and cimetidine treatment, the intercellular clefts became extremely short and devoid of projections. The tubular system filled the cytoplasm adjacent to the luminal surface (Fig. 1 *c*). The cytoskeletal matrix framed a cortex interposed between the tubular system and the luminal plasma membrane (Fig. 1 *d*).

ATPase, K-NPPase, and Marker Enzyme Activities in Chicken Gastric Glands: Effect of Oxyntic Cell Secretory State

ATPase activity in total homogenates and microsomal fractions was assayed under three experimental conditions: no K^+ added (Mg-ATPase activity), K^+ alone, and K^+ plus valinomycin.

The Mg-ATPase activity and its sensitivity to K^+ and valinomycin changed according to the secretory state of gastric glands. In total homogenates from resting glands, K-ATPase was not detected even in the presence of valinomycin. In microsomal fractions, K^+ induced a 10% increase over the basal activity, whereas valinomycin produced a 20% additional activation. In secreting glands, K-ATPase activity was detected consistently both in total homogenates and in microsomes, representing a 20% increase over the basal activity. In this case valinomycin was slightly inhibitory.

In general, K-ATPase activity was extremely labile and vanished after freezing or aging at 4°C. On the other hand, Mg-ATPase was very active and stable, remained unchanged for several months at −20°C, and was not affected by aging at 4°C, hypoosmotic shock, or sonication.

The cytochemical reaction to detect Mg-ATPase on frozen sections of gastric glands (Fig. 2, *a* and *b*) shows that the enzyme is located at the luminal cytoplasm of resting oxyntic cells (Fig. 2 *a*). Stimulation induced a change in localization of the Mg-ATPase reaction product, which then outlined the intercellular cleft of the apical pole of oxyntic cells (Fig. 2 *b*). The drastic change induced in the cellular distribution of the Mg-ATPase by the onset of HCl secretion is identical to that found for K-NPPase (Fig. 2 *c* and *d*) (Koenig, 1984).

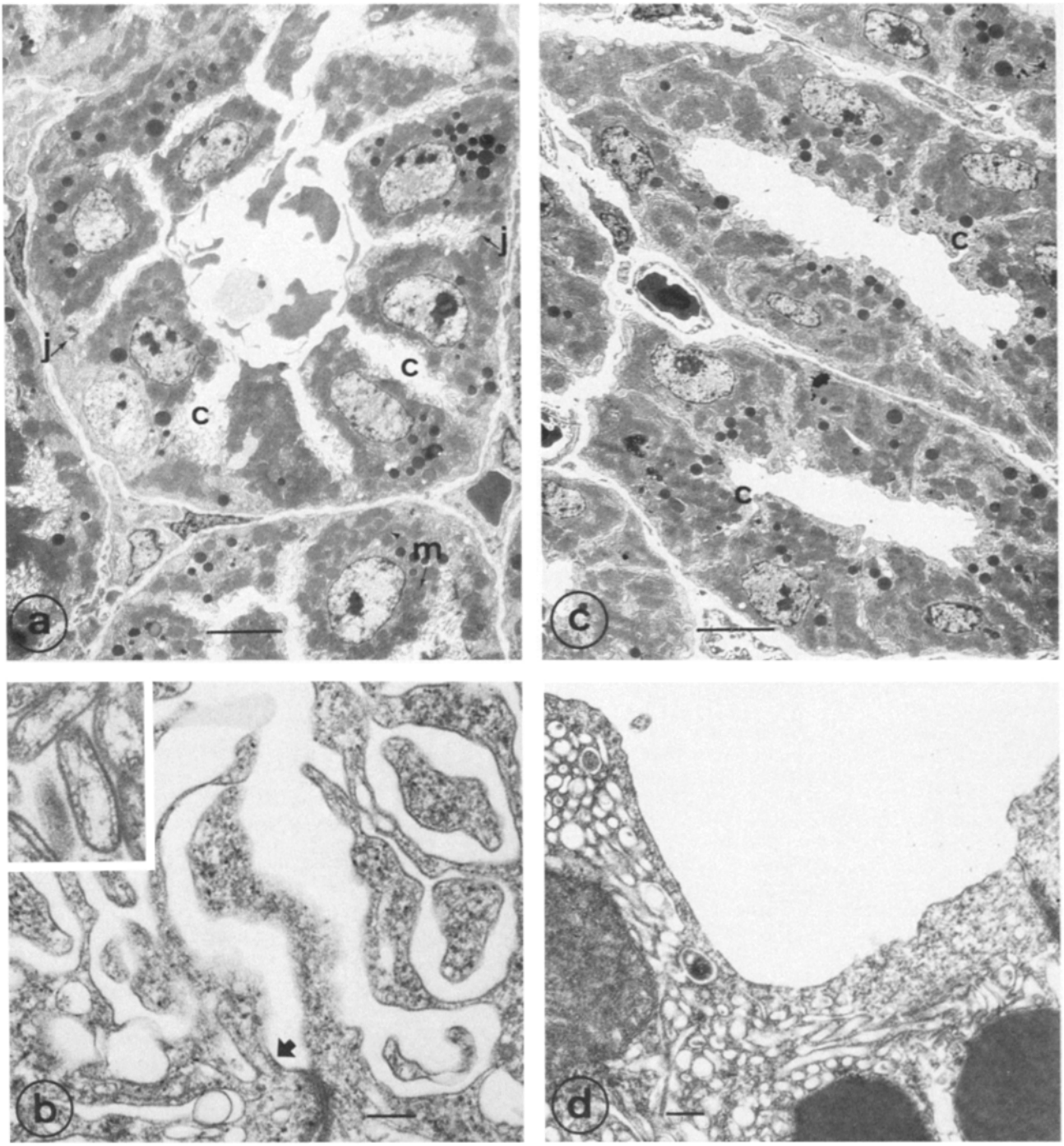

Figure 1. Ultrastructure of chicken oxyntic cells in resting and HCl-secreting glands. (*a*) Cross section through a glandular tubule, after histamine stimulation. Deep intercellular clefts (*c*) surround the secretory pole. Cytoplasmic processes project from these clefts, especially toward the junctional complex (*j*). Mitochondria (*m*) fill the cytoplasm. Zymogen granules are also present. (*b*) Intercellular cleft in a histamine-stimulated oxyntic cell. Irregular cytoplasmic processes containing microfilaments and a dense granular matrix fill the clefts. When sectioned at an appropriate angle, associations between microfilaments and membrane are observed (*arrow*). (*c*) Cross section through gland tubules in resting state. The intercellular clefts (*c*) are very short and the luminal surface is smooth. The apical cytoplasm contains the tubular system. (*d*) Luminal surface of resting oxyntic cells. The tightly packed tubules form a feltwork that excludes most of the cytoplasmic matrix. A cortex is seen between the tubular system and the luminal plasma membrane. Bars: (*a* and *c*) 5 μm; (*b* and *d*) 0.2 μm.

This finding suggests that at least part of the Mg-ATPase activity seems to be related to an enzyme located in the membranous systems of the oxyntic cell's secretory pole.

In contrast to K-ATPase, the associated K-NPPase activity was easily detected in homogenates of resting or HCl-secreting chicken gastric glands. After addition of K^+, the basal NPPase activity in homogenates increased by 75%. In microsomal fractions this increase reached 200%. The

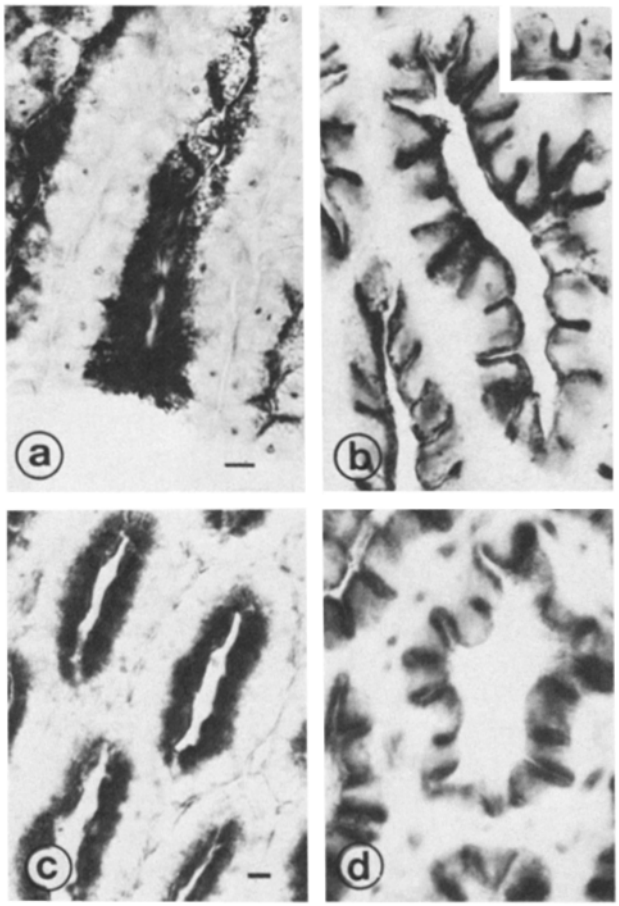

Figure 2. Cytochemical staining for Mg-ATPase and K-NPPase in chicken gastric glands. (*a–b*) Mg-ATPase. Fixation was carried out in 3% glutaraldehyde, 0.1 M phosphate buffer, pH 7.2. Frozen sections were incubated in the reaction medium containing 50 mM Tris-glycylglycine buffer, pH 7.0, 1 mM MgCl, 4 mM ATP, and 3.6 mM $Pb(NO_3)_2$ for 5 min at room temperature. Sections were rinsed in distilled water, treated with 1% yellow ammonium sulfide, and mounted in glycerine jelly. (*a*) Glandular tubule in resting state induced by cimetidine. The Mg-ATPase reaction product stains only the secretory pole of oxyntic cells and is concentrated in the luminal cytoplasm. (*b*) Glandular tubule after stimulation by histamine. The distribution of Mg-ATPase reaction product drastically changes. The intercellular clefts appear heavily stained and the secretory pole of each oxyntic cell is clearly outlined. In the inset the concentration of reaction product in the deeper zone of the intercellular clefts is clearly visualized. (*c–d*) Frozen sections after 30 min incubation in the standard medium for gastric K-NPPase (Koenig, 1984). In glandular tubules in resting state, after cimetidine (Fig. 3 *c*), the K-NPPase reaction product localized in the luminal oxyntic cells cytoplasm. In the histamine-stimulated glands (Fig. 3 *d*), the K-NPPase cytochemical reaction outlined the secretory pole of oxyntic cells. It stained the rim of cytoplasm adjacent to the intercellular clefts. Bars, 10 μm.

K-NPPase activity was not affected by hypoosmotic shock or the presence of valinomycin, and remained stable during several days at 4°C and for months at −20°C. Based on these results and previous cytochemical studies (Koenig, 1984; Fujimoto et al., 1986) K-NPPase was chosen as a marker enzyme for the membranous system of oxyntic cells during quantitative subcellular fractionation.

Table I. Absolute Values of Enzymes and Proteins in Homogenates from Resting and Stimulated Chicken Gastric Glands

		Absolute values	
	n	Resting glands	Stimulated glands
Cytochrome oxidase	5	46.21 ± 9.63	49.71 ± 9.70
Catalase	4	1.03 ± 0.06	1.07 ± 0.28
Acid phosphatase	3	3.69 ± 0.61	3.58 ± 0.93
NADPH-cytochrome *c* reductase	4	0.54 ± 0.12	0.49 ± 0.08
5′-nucleotidase	4	1.66 ± 0.58	2.13 ± 0.41
Phosphoglucomutase	2	11.01 ± 1.05	9.18 ± 0.82
(K^+-EDTA) ATPase	2	0.58 ± 0.03	0.77 ± 0.06
K^+-NPPase	4	4.47 ± 0.24	1.64 ± 0.61*
Mg^{++}-ATPase	4	142.90 ± 32.53	238.80 ± 26.03‡
Proteins	4	172.17 ± 20.87	179.40 ± 29.53

Absolute values refer to 1 g fresh weight of gastric glands. They are given as means ± SD, in units for enzymes and in milligrams for protein.
* Difference from resting glands significant at $P < 0.006$.
‡ Difference from resting glands significant at $P < 0.001$.

The values of the absolute enzymatic activities and total protein in homogenates of resting and secreting gastric glands are listed in Table I. A drastic change was found in the absolute units of K-NPPase and Mg-ATPase as a result of histamine stimulation. K-NPPase activity decreased to one-third of that observed in resting glands, whereas the activity of Mg-ATPase was almost doubled. No significant differences were observed in the activities of the other enzymes.

Differential Centrifugation

The distribution patterns and the recoveries obtained in the fractionation of resting and HCl secreting gastric glands are listed in Table II. To draw the distribution profiles shown in Fig. 3, data were corrected for recovery as described by de Duve et al. (1955).

The distribution of cytochrome oxidase expresses the behavior of mitochondria in the fractionation. About 90% of this enzyme was recovered in the NM fraction. P fraction contained <4% of cytochrome oxidase. In stimulated glands, a small but significant increase in the average of cytochrome oxidase recovered in NM fractions was observed. NADPH-cytochrome *c* reductase, a microsomal marker, was concentrated in L and P fractions, with a high proportion in S fraction. This distribution was different from that found in liver, where the enzyme has very low soluble activity (Beaufay et al., 1974). Over 50% of catalase units were concentrated in the L fraction. In contrast to what has been described in liver, a low amount of this enzyme was present in the supernatant. This might be due to a lesser fragility of gastric peroxisomes. A small but significant increase of catalase activity in the S fraction was observed in stimulated glands. Acid phosphatase, the lysosomal marker, was concentrated in the L fraction. The conventional plasma membrane marker 5′-nucleotidase was mainly distributed in fractions NM and P. When N and M fractions were separated, the enzyme remained associated mainly to N, as it does in liver (Beaufay et al., 1974). The distribution of (K-EDTA)-ATPase showed that, although most of the myosin sedimented in fraction S, over 25% of the enzyme was associated to fractions L and P.

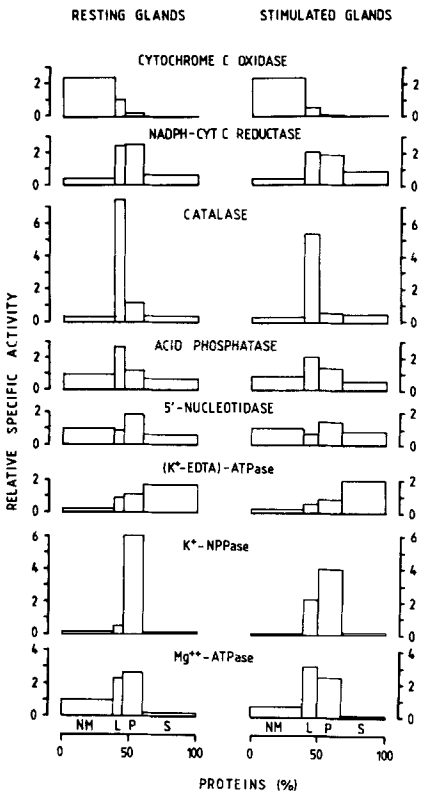

Figure 3. Distribution pattern of constituents after fractionation of resting and stimulated chicken gastric glands by differential centrifugation. Graphs were constructed from the data given in Table II. Fractions are plotted in the order of the average coefficient of sedimentation of their subcellular components, i.e., from left to right: NM (nuclear + heavy mitochondrial), L (light mitochondrial), P (microsomal), and S (supernatant) fractions. Each fraction is represented separately in the ordinate scale by the relative specific activity of the constituent (percentage of total amount per percentage of total proteins recovered in the fraction). In the abscissa scale each fraction is represented accumulatively by its percentage of protein.

The distribution of K-NPPase displayed a remarkable change as regards the gastric gland functional state. Fraction P of resting glands concentrated more than 90% of K-NPPase units, with a relative specific activity over 6. Stimulation by histamine induced a change in the distribution pattern of the enzyme. In fraction P the activity was reduced to ~70%, with a concomitant increase of 20% in fraction L. This behavior can be explained by a change in the sedimentation pattern of the vesicles containing the enzyme.

However, since we have shown that the K-NPPase total activity of the homogenate decreases by ~65% with stimulation (Table I), a differential decrease of enzyme activity in the P fraction of stimulated glands could have occurred. Nevertheless, no significant increase in the relative specific activity of this enzyme was found in NM and S fractions. Calculations based on the percent of K-NPPase activity in fractions from resting glands (Table II) and on the total K-NPPase activities (Table I) demonstrate that the activity in the L fraction could have increased at most up to 10%, while we found that it increased by more than 22%. This shows that the percentage of enzyme activity only increases in L fraction, and that this increase is higher than that predicted by a differential decrease in enzyme activity in the P fraction. This, taken together with the cytochemical redistribution of K-NPPase already presented (Fig. 2, *c* and *d*), suggests that membranes with K-NPPase activity that concentrated in P fraction of resting glands sediment in the L fraction of stimulated glands.

The changes in functional state of gastric glands were also related to modifications in the distribution pattern of Mg-ATPase. Most of the activity of this enzyme, both in resting and secreting glands, was concentrated in L and P fractions. A significant amount of activity was also found in the NM fraction, apparently associated with mitochondria. When N and M fractions were isolated, activity was concentrated in M fraction (not shown).

In stimulated glands, the percent of Mg-ATPase activity sedimenting in the NM fraction decreased significantly, while it increased in the L fraction. This phenomenon may be related to the almost twofold increase of the enzyme's absolute activity in secreting glands (Table I). Estimations based on the percent of Mg-ATPase activity in fractions from resting glands (Table II) and on the total Mg-ATPase activities (Table I) showed that, although upon stimulation the relative specific activity in the NM fraction decreased, the specific activity (expressed as units of Mg-ATPase per milligrams of protein) remained constant. At the same time, the specific activity of Mg-ATPase sedimenting in fractions L and P increased 3.4- and 1.75-fold respectively. This suggests that changes in the distribution pattern of Mg-ATPase, associated with the gland functional state, are the consequence of a specific increase in the Mg-ATPase sedimentation in the L and P fractions. Based on these results and on the cytochemical redistribution of Mg-ATPase (Fig. 2, *a* and *b*), it can be proposed that membranes with nonmitochondrial Mg-ATPase activity that concentrate in P fraction of resting glands sediment in the L fraction of stimulated glands.

Finally, the distribution pattern of proteins changed with the gland secretory state. Proteins present in the L fraction of stimulated glands increased significantly, while they decreased in the S fraction (Table II, Fig. 3).

Morphological Examination of L and P Fractions

The high ratio of K-NPPase activity in the P fraction of resting glands agrees well with their enrichment in membranous smooth vesicles (Fig. 4 *a*), presumably generated from the tubular system of oxyntic cells (Fig. 1, *c–d*). In L and P fractions of stimulated glands, the change in sedimentation properties of K-NPPase was associated with the presence of heterogeneous membrane profiles that enclosed cytoplasmic matrix (Fig. 4 *b*). Such profiles may correspond to fragments of the cellular processes present in stimulated oxyntic cell secretory surface (Fig. 1, *a–b*).

In P fractions of resting glands the distinctive membrane structure of the tubular system was seen after tannic acid staining (Fig. 5). The unit membrane of the vesicles showed a thick inner dense lamina. Most vesicles presented their cytoplasmic side to the medium, but some of them were inversely sealed. Occasionally, pentalaminar membranes were seen between closely associated vesicles, as well as cytoplasmic fragments enclosing a poorly structured matrix and membranous vesicles.

In P fractions of stimulated glands, the abundant cytoplasmic fragments contained microfilaments apparently associ-

Table II. Enzyme Distribution after Differential Centrifugation of Chicken Gastric Glands in Resting and Secreting HCl States

	Functional state	n	NM	L	P	S	Recovery
Proteins	R	4	37.8 ± 1.1	7.1 ± 1.8	15.0 ± 2.7	39.9 ± 0.7	101.2 ± 7.1
	S	4	39.1 ± 2.0	10.5 ± 1.8*	17.3 ± 3.7	33.0 ± 2.1*	101.3 ± 5.6
Cytochrome oxidase	R	5	88.2 ± 2.6	6.8 ± 2.0	3.2 ± 2.2	1.7 ± 1.6	99.1 ± 6.0
	S	5	91.8 ± 1.5*	5.8 ± 0.5	1.9 ± 1.4	0.8 ± 0.7	108.9 ± 12.5
NADPH-cytochrome *c* reductase	R	4	17.5 ± 4.1	17.2 ± 6.7	36.6 ± 6.8	28.4 ± 0.4	108.9 ± 11.9
	S	4	17.4 ± 2.6	22.1 ± 5.2	33.0 ± 4.0	28.5 ± 4.9	115.5 ± 3.5
Catalase	R	4	14.8 ± 2.9	53.2 ± 8.9	16.8 ± 7.8	15.1 ± 0.9	103.5 ± 5.8
	S	4	14.4 ± 2.0	56.7 ± 1.2	10.2 ± 1.1	18.7 ± 1.7*	99.9 ± 10.5
Acid phosphatase	R	3	36.2 ± 7.4	18.8 ± 3.7	18.3 ± 6.5	26.8 ± 16.0	98.2 ± 10.9
	S	3	34.8 ± 4.9	21.4 ± 2.9	24.3 ± 7.6	19.6 ± 12.2	106.9 ± 8.9
5'-nucleotidase	R	4	37.5 ± 2.0	6.6 ± 1.0	28.1 ± 3.9	27.8 ± 4.4	92.8 ± 9.6
	S	4	20.9 ± 7.0	7.2 ± 2.5	24.9 ± 5.5	27.1 ± 0.9	88.8 ± 15.7
(K^+ + EDTA) ATPase	R	2	9.6 ± 1.8	6.4 ± 1.8	16.3 ± 3.2	67.6 ± 8.3	114.7 ± 9.1
	S	2	12.4 ± 0.9	6.5 ± 0.4	14.2 ± 3.2	66.8 ± 12.2	108.7 ± 5.1
K^+-NPPase	R	4	2.0 ± 1.7	3.7 ± 1.2	90.3 ± 4.3	4.0 ± 2.8	108.0 ± 12.5
	S	3	2.1 ± 1.0	22.5 ± 3.1*	69.5 ± 6.0*	6.0 ± 2.9	109.1 ± 12.1
Mg^{++}-ATPase	R	4	36.4 ± 5.4	15.9 ± 7.3	39.8 ± 9.3	7.8 ± 2.9	102.3 ± 8.1
	S	4	23.4 ± 0.7*	32.4 ± 11.3*	42.0 ± 11.2	2.2 ± 0.5*	103.8 ± 4.6

Results are given as means ± SD, in percentage of the recovered total activity.
* Statistically significant difference with respect to the same fractions from resting glands.

ated with the membrane (Fig. 6). After tannic acid staining, the plasma membrane of the fragments showed a thick inner lamina. Their structure was comparable to that of the apical processes of stimulated oxyntic cells (Vial, 1982).

Fractionation of Postmitochondrial Supernatants by Chromatography in Sepharose 2B

The biochemical results presented raise several issues. First, the finding of membrane marker enzymes in S fractions (25% of NADPH-cytochrome *c* reductase and 5'-nucleotidase and 8% of K-NPPase and Mg-ATPase) suggests that some particulate components did not sediment by centrifugation. Second, the low or nonexistent activity of K-ATPase in homogenates raises the question that avian gastric glands may have a pool of K^+ responsible for endogenous stimulation of the basal Mg-ATPase activity. Quantification of K^+ content in homogenates by flame photometry gave values of ~130 meq/liter. This figure decreased to 10% of the initial value after dialysis of the homogenates. Finally, the decrease in protein content of the stimulated gland S fraction might be the result of soluble proteins trapped by membrane fragments.

Isolation of the K-NPPase-containing vesicles by exclusion chromatography of postmitochondrial supernatants on Sepharose 2B was used to clear up these issues. This technique gives a better separation between the soluble components and the organelles and vesicles.

Fig. 7 shows the elution profiles of proteins and marker enzymes after filtration of postmitochondrial supernatants from stimulated glands on Sepharose 2B. The distribution profile of cytochrome oxidase sharply defined the particulate fraction, which corresponded to the sum of L and P fractions from differential centrifugation (LP-Sepharose fraction). In turn, the soluble fraction was defined by the distribution of phosphoglucomutase.

A high Mg-ATPase activity persisted in LP-Sepharose fractions. The sensitivity to K^+ stimulation was slightly increased. Besides, a significant and reproducible activation was observed in the presence of K^+ and valinomycin. K-NPPase, Mg-ATPase, and K-valinomycin-ATPase followed exactly the distribution of cytochrome oxidase. No activity of these enzymes was found in the soluble fractions. On the other hand, NADPH-cytochrome *c* reductase and 5'-nucleotidase showed that the soluble fraction contained a proportion of these enzymes similar to that found after differential centrifugation.

It is noteworthy that no activity of the soluble marker phosphoglucomutase was found in the particulate LP-Sepharose fraction. If the decrease in proteins in the soluble fraction of stimulated glands observed in differential centrifugates had resulted from trapping of soluble proteins by sedimenting membrane vesicles, it might be expected that at least a small amount of phosphoglucomutase would be associated with particles. Since phosphoglucomutase was not found in the particulate LP fraction, the decrease in soluble proteins observed in stimulated glands after differential centrifugation would correspond to specific proteins. The morphological evidence already presented in Fig. 6 suggests that part of them correspond to cytoskeletal proteins.

Elution profiles similar to those shown in Fig. 7 were obtained when resting gland postmitochondrial supernatants were filtered on Sepharose 2B. A reproducible activation of Mg-ATPase by K^+ and valinomycin was also observed in the particulate LP-Sepharose fraction of resting glands. The absolute values and percentage of recovered activities of enzymes and proteins were quite similar in LP-Sepharose fractions obtained from stimulated and resting glands. However,

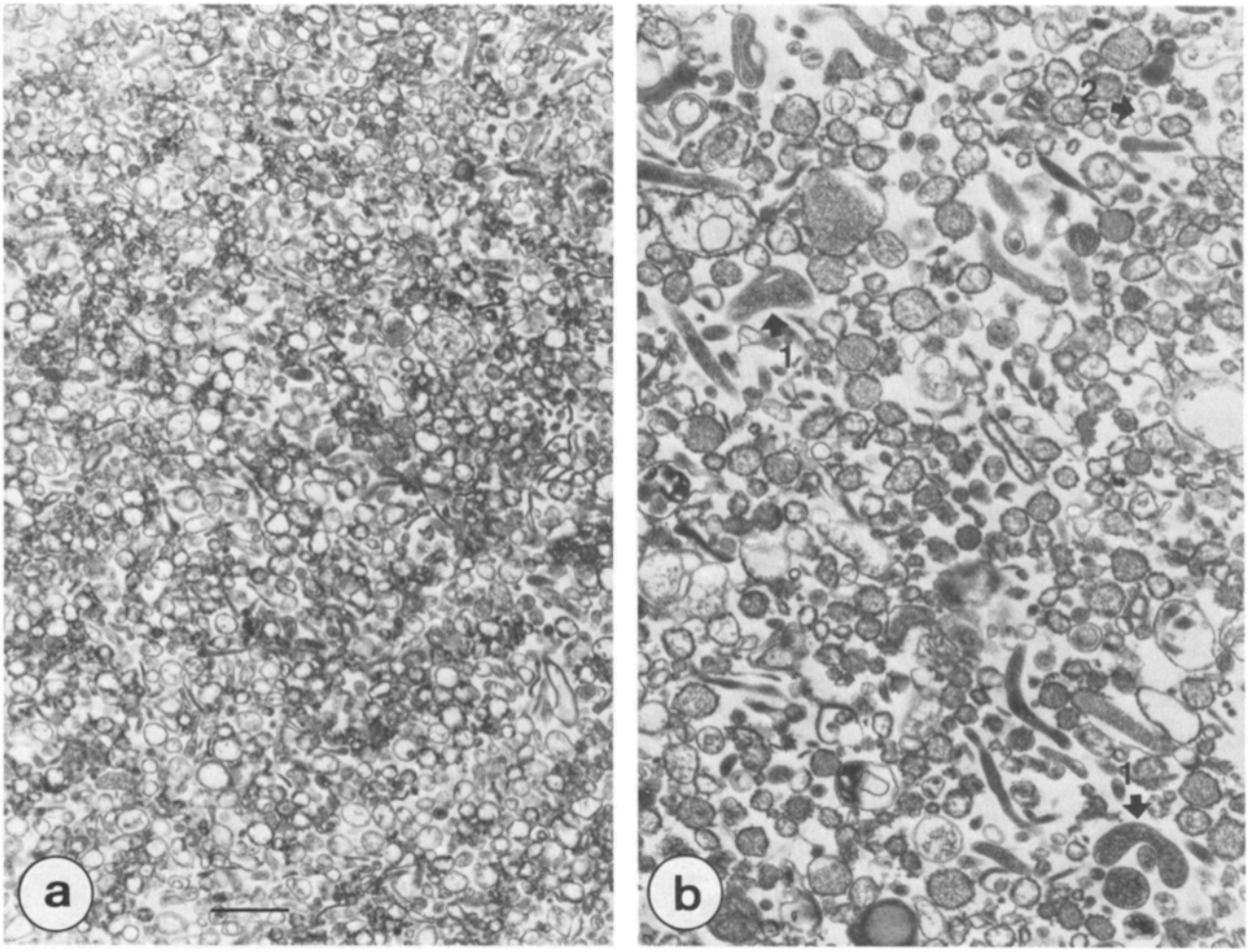

Figure 4. Electron micrographs of P fractions isolated from resting and stimulated chicken gastric glands. (*a*) The P fraction from resting glands consists mainly of smooth membranous vesicles with diameters between 0.1 and 0.2 μm. (*b*) The P fraction from stimulated glands contains elongated membranous profiles with diameters between 0.2 and 0.4 μm. A dense granular and filamentous material, and occasionally small vesicles, are seen inside the membranous profiles (*arrow 1*). A few free small vesicles like those in Fig. 4 *a* are also observed (*arrow 2*). Bar, 0.5 μm.

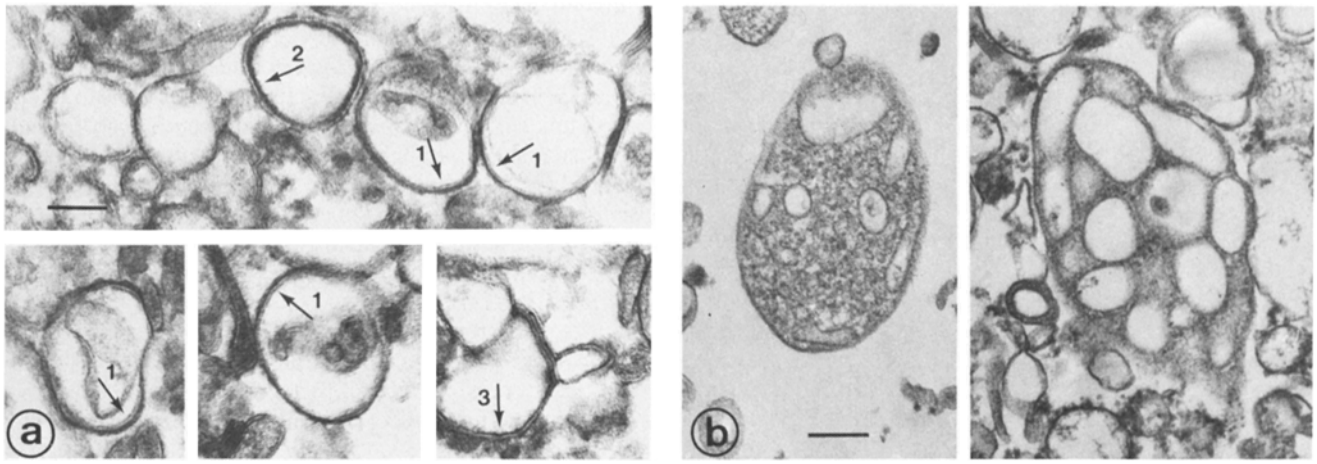

Figure 5. A high magnification electron micrograph showing the distinguishing features of the membranes present in the P fraction of resting glands in samples stained with tannic acid. (*a*) Membrane vesicles similar to those observed in Fig. 4 *a*. Most of them show the thickening of the cytoplasmic sheet of the unit membrane (*arrow 1*). Some vesicles with an opposite orientation are occasionally seen (*arrow 2*). A dense material appears associated with the external surface of some vesicles (*arrow 3*). (*b*) Membrane-surrounded structures showing a heterogenous content, corresponding either to vesicles (*left*) or to a poorly structured matrix (*right*), are observed. Bars: (*a*) 0.1 μm; (*b*) 0.2 μm.

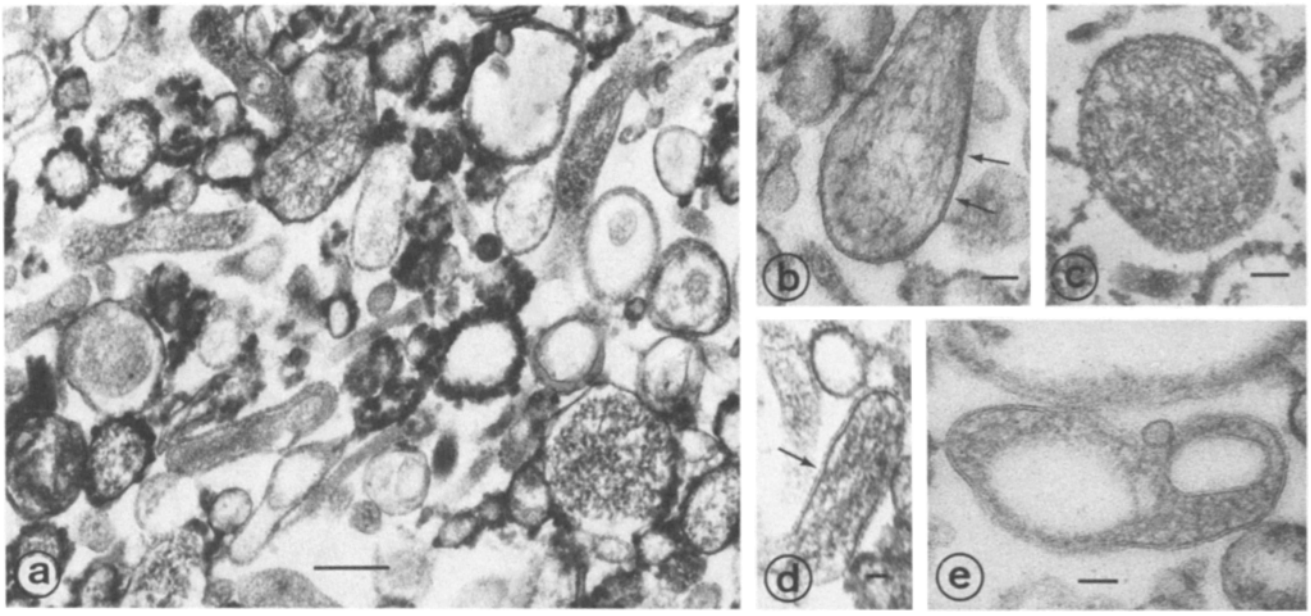

Figure 6. Details of the membranous profiles present in fraction P of stimulated glands after tannic acid staining. Microfilaments are clearly seen inside the membrane-surrounded profiles. (*a*, *c*, *d*, and *e*). When sectioned at an appropriate angle, the thickening of the cytoplasmic sheet of the membrane is observed. Connections between the cytoplasmic membrane and microfilaments are seen (*arrows*). Occasionally, vesicles surrounded by a filamentous matrix were found inside these profiles (*e*). Bars: (*a*) 0.2 μm; (*b–e*) 0.05 μm.

the difference in activity of Mg-ATPase and K-NPPase found in resting and HCl secreting gland homogenates persisted. For K-NPPase, the functional state of the glands had no effect upon the sensitivity to K^+ concentration. The lower activity of K-NPPase in LP-Sepharose fractions of stimulated glands was not modified by valinomycin or deoxycholate. Yet, the sensitivity of the enzyme to substrate concentration changed in relation to the gland secretory state. Upon stimulation, a slight but significant difference was found between the K_m and the V_{max} of K-NPPase: K_m increased from 1.75 to 4.75 and V_{max} changed from 0.135 to 0.130. The kinetic parameters of basal Mg-ATPase were not affected by the gland secretory state.

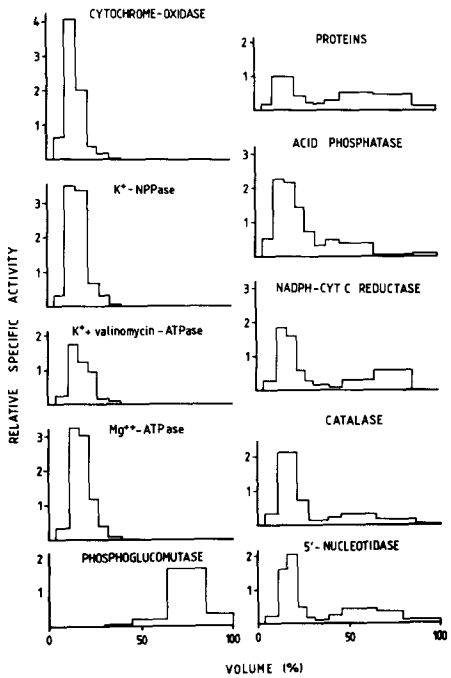

Figure 7. Elution profiles of proteins and enzymes from stimulated gastric gland postmitochondrial supernatant. Filtration was carried out in a Sepharose 2B column (2.5 × 5.0 cm) equilibrated with 0.25 M sucrose, 3 mM imidazole-HCl, pH 7.4. Ordinates represent the relative concentration of each component (units/ml per C, where C is the total amount of activity recovered, divided by the total recovered volume). In the abscissa each fraction is represented cumulatively by its percentage of the total volume eluted. The recovered activities varied between 80 and 120%.

Subfractionation of LP-Sepharose Fractions by Centrifugation in Linear Density Gradients of Metrizamide

The density distribution of enzymes and proteins after isopycnic equilibration of LP-Sepharose fractions of resting and stimulated glands is shown in Fig. 8. The distribution of K-NPPase demonstrated that fractionation in metrizamide gradients clearly resolved the tubular system from microsomes (NADPH–cytochrome *c* reductase) and mitochondria (cytochrome oxidase), the two other main components of the LP-Sepharose fraction, on a protein basis. This fractionation method also clearly separated gastric peroxisomes from the other organelles.

After subfractionation of LP-Sepharose fractions from resting glands the distribution of K-NPPase was clearly bimodal. A high proportion of the enzyme equilibrated between densities of 1.06–1.08 g/ml, with a second peak at a density of ~1.125 g/ml. Stimulation induced a drastic change in this distribution pattern and most of the K-NPPase appeared to be associated with membranes equilibrating at a density of 1.12 g/ml or higher. This change was quantitatively represented by an increase in the median equilibrium density of K-NPPase from 1.0878 ± 0.0136 g/ml in resting glands, to 1.1310 ± 0.0129 g/ml in stimulated glands. The median

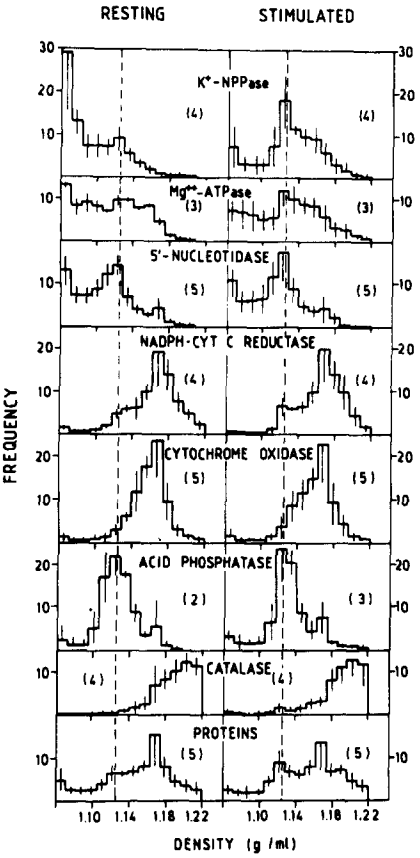

Figure 8. Distribution of enzymes after fractionation of LP-Sepharose fractions of gastric glands in resting and HCl-secreting states in continuous metrizamide density gradients. Standardized and average results are shown. Ordinates represent average frequency of the components of each fraction, $Q/\Sigma Q \cdot \Delta\rho$, where Q represents the activity found in the fraction, ΣQ the total activity recovered, and $\Delta\rho$ the increment in density from top to bottom of the gradient in the centrifuge tube. Frequency is plotted against density. Numerals in parenthesis refer to number of experiments. Vertical lines through histogram bars represent SD of frequency. The demarcation line at density 1.125 allows easier comparison of distribution profiles. The recovery of enzymes and proteins varied between 75 and 130%.

equilibrium densities and the distribution profiles of the other marker enzymes assayed remained unaltered, except for Mg-ATPase and total protein. Although no significant difference was observed in the median equilibrium density of total protein, the change in the distribution of K-NPPase correlated with a change in the distribution of protein, which was found to be bimodal in stimulated glands. The protein peak at 1.125 g/ml seems to correspond to the increase in the K-NPPase equilibrated at this density. A small amount of NADPH–cytochrome *c* reductase also appeared to be associated with the main peak of K-NPPase in stimulated glands.

K-ATPase activity was assayed in some of the experiments pooled in Fig. 8. The distribution of this enzyme was quite similar to that of K-NPPase. Mg-ATPase partly followed the distribution of K-NPPase. In gradients of stimulated glands, an apparent decrease of Mg-ATPase was found at the low density region of the gradient and an increase at a density of 1.125 g/ml. Judging by the behavior of cytochrome oxidase, only a small proportion of Mg-ATPase appeared to be localized in mitochondria.

The specific activity of K-ATPase and its sensitivity to valinomycin were determined in the low density fractions of the gradient in one of the paired experiments shown in Fig. 8. Results are presented in Table III. The specific activity of Mg-ATPase found in fractions of stimulated glands was about three times higher than that detected in resting glands. The activation of Mg-ATPase by K^+ (K-ATPase), was 17-fold greater in membranes of stimulated glands, whereas the enzyme activity in the presence of valinomycin was only 1.3-fold greater than that in membranes of resting glands. It is noteworthy that membranes isolated from resting glands in the low density region of the gradient showed a purification higher than that of stimulated glands (70 and 20% of K-NPPase total activity, respectively). It is thus possible that the increase could have been even higher in Mg-ATPase and K-ATPase specific activities in membranes of stimulated glands. On the other hand, in the absence of valinomycin, almost no activity of K-ATPase was detected in membranes of resting glands, whereas in membranes of stimulated glands, a 1.7-fold increase of the activity was induced by the ionophore.

Polypeptide Composition of Membrane Fractions Purified by Isopycnic Centrifugation in Metrizamide Gradients

The SDS-PAGE polypeptide pattern of fractions obtained by isopycnic centrifugation of LP-Sepharose preparations from resting and stimulated glands is shown in Fig. 9. Distribution of the enzymes in these experiments was similar to that in Fig. 8. In resting glands, ~70% of the K-NPPase units was concentrated in the light density peak corresponding to fraction 1. This fraction was enriched in polypeptides of 250, 180, 130, 95, 48, 40, 34, 30, and 21 kD. In stimulated glands, ~20% of the K-NPPase units was associated with fractions 1 and 2 of the light density region of the gradient whereas 65% was present in fractions 6 to 8, the heavy density peak of K-NPPase units. Only slight differences were observed in the polypeptide patterns of the heavy density peak fractions of resting and stimulated glands when polyacrylamide gels loaded with similar amounts of protein were analyzed by densitometric scanning.

In contrast, major differences were observed in the patterns of the light density peaks of K-NPPase activity (Fig. 10). The main changes were observed in polypeptides of 250, 200, 180, 160, 95, 43, 40, and 28 kD. Polypeptides of 250 kD (which co-migrated with filamin), 180 kD (which co-mi-

Table III. Specific Activity of K-ATPase in Purified Membranes from Stimulated and Resting Glands: Effect of Valinomycin

	Resting glands	Stimulated glands
No additions	0.092	1.576
Plus valinomycin	2.064	2.697

Enzymatic activities are expressed in μmol/min per mg of protein. They were assayed in membranes equilibrating at density 1.06–1.08 g/ml in metrizamide gradients (see Fig. 8), which contained respectively 70 (resting) and 20 (stimulated) % of the total K-NPPase activity in the gradient.

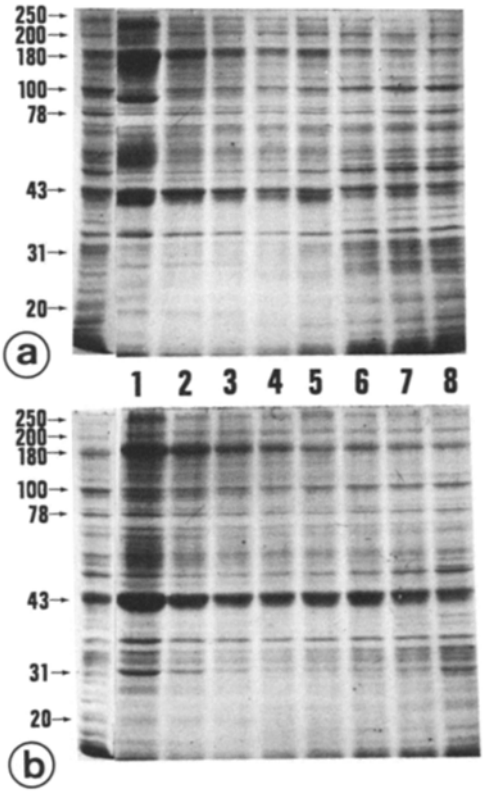

Figure 9. Polyacrylamide gels of fractions obtained by isopycnic fractionation of LP-Sepharose fractions of resting and stimulated glands. Equivalent volumes (30 µl) of undiluted samples from the fractions were loaded onto the gel. The first lane at the left corresponds to the original LP-Sepharose sample. Lanes *1–8* correspond to fractions from the top to the bottom of the tube in fractionation experiments similar to those presented in Fig. 8. Lane *1* corresponds to the lowest density fraction containing the light density peak of K-NPPase activity, whereas lanes *6* to *8* correspond to the heavy density peak of this enzyme activity. (*Top*) Fractions of resting glands; (*bottom*) fractions of stimulated glands.

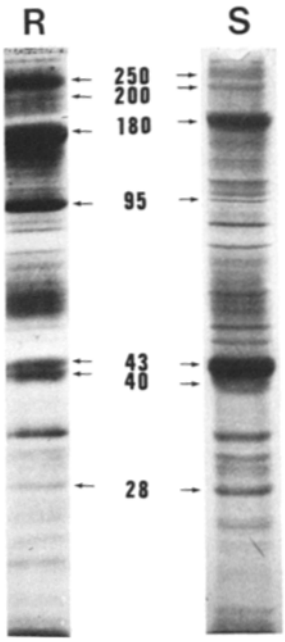

Figure 10. Comparison of the polypeptide patterns of the light density fractions of resting and stimulated glands obtained from metrizamide gradients. Equivalent amounts of protein (30 µg) from the lowest density fraction (fraction 1, see Fig. 9) were loaded onto the gel.

grated with clathrin), 130–160 kD, 95 kD, equivalent proportions of polypeptides of 43 kD (which co-migrated with actin), and 40 kD were found in fraction 1 of resting glands. This fraction showed a lower proportion of polypeptides of 28 and 200 kD (which co-migrated with myosin). Membranes present in fraction 1 of stimulated glands were characterized by a decrease in their association with polypeptides of 250, 180, 130–160, 95, and 40 kD. An increase in the percentage of polypeptides of 200, 43, and 28 kD was observed.

As already noted, ~70% of the enzymatic marker of the secretory pole membranes was present in the light membrane fraction of resting glands, while the membrane fraction of stimulated glands contained only 20% of the K-NPPase units. No changes were observed in the remaining marker enzymes. This result shows that different amounts of membranes derived from the secretory pole of oxyntic cells were equilibrated in this fraction. It therefore seemed interesting to analyze the proportion of polypeptides that coequilibrated in this fraction as a function of the concentration of membranes.

The percentage of K-NPPase units was 3.3-fold higher in the membrane fraction of resting glands. With this in mind, we quantitatively analyzed SDS-PAGE gel profiles. A marked increase was found in the association of 200-, 43-, and 28-kD polypeptides with the membrane fraction from stimulated glands; in turn, a striking decrease occurred in the association of 250- and 95-kD polypeptides.

No significant data were obtained from the analysis of the polypeptide composition of the fraction containing the heavy density peak of K-NPPase activity. This result must be ascribed to a more heterogeneous organelle composition of this fraction.

Discussion

A comparative analysis of resting and histamine-stimulated chicken gastric glands by quantitative subcellular fractionation is described. Our results show that specific modifications in the membrane fractions derived from oxyntic cell secretory pole are related to the HCl secretory state.

Two considerations give support to the experimental approach. First, homogeneous populations of chicken oxyntic cells were obtained from stomachs in resting or secretory state. Despite anoxia (Vial and Orrego, 1963) and gland manipulation at low temperature, the morphology of each secretory state persisted until homogenization. Second, practically the entire population of K-NPPase–containing vesicles was used in each experiment, avoiding possible artifacts due to selection of membrane populations by successive fractionation.

A clear correlation was found between changes in the isolated membranes of the secretory pole and the morphological and functional reorganization of oxyntic cells and the quantitative biochemical changes in the isolated membranes of the secretory pole. The sedimentation pattern of K-NPPase, marker of the apical pole membranes, was modified. At the same time, a change was observed in the total activities of K-NPPase and Mg-ATPase, apparently mediated by modifications in the biochemical properties of the secretory pole

membranes. When membrane vesicles were disrupted by physical methods, no modification of the activity characteristic of each functional state was observed. This result shows that the orientation of the vesicular elements has no influence on the measured activities of K-NPPase and Mg-ATPase. These results agree with those reported in sealed vesicles from mammalian gastric glands by Saccomani et al. (1977). On the other hand, the twofold increase in K-NPPase activity, observed in total homogenates and LP-Sepharose fraction of resting chicken oxyntic cells assayed under hypoosmotic conditions cannot be explained as a result of the 50% latency, reported by Hirst and Forte (1985), in rabbit gastric vesicles.

Clear differences were found in the affinity of K-NPPase for its substrate when comparing membranes of resting and stimulated glands by kinetic analysis of K-NPPase. The change in K_m can be attributed to modifications in the protein itself, induced by changes in the association of subunits, union of specific ligands, or modifications in lipid environment.

Subcellular fractionation of chicken gastric glands in the resting state resulted in more than 90% recovery of K-NPPase units in the microsomal fraction. These microsomes showed a functional characteristic typical of the microsomes from mammalian resting glands, i.e., low K^+ permeability as indicated by valinomycin dependence for K^+ activation of ATPase (Saccomani et al., 1977; Wolosin and Forte, 1981*a*; Ljungstrom et al., 1984*a*; Hirst and Forte, 1985). Purification of the LP-Sepharose fractions of resting glands by density gradient centrifugation yielded a vesicular fraction, mostly vesicles from the tubular system, which contained 70% of the units of the marker enzyme of the secretory pole membranes.

The redistribution of K-NPPase observed after fractionation of histamine-stimulated gastric glands is in agreement with the replacement of the small sealed vesicles by denser and heterogeneous membranous profiles of variable size. These profiles enclosed microfilaments, cytoplasmic matrix, and some small vesicles, apparently cytoplasmic fragments structurally analogous to the cytoplasmic processes of the expanded secretory surface. These vesicular elements showed a great dispersion in the density gradient.

The redistribution of K-NPPase upon stimulation was specific; no significant changes in the distribution of other organelle markers were observed in density gradient centrifugation experiments. During fractionation, only proteins and nonmitochondrial Mg-ATPase followed the K-NPPase redistribution.

The distribution of K-NPPase after fractionation defined the tubular system of oxyntic cells as an organelle with unique biochemical and physicochemical properties different from those of endoplasmic reticulum, mitochondria, and plasma membrane. The sedimentation properties of this membranous system changed when the organization of the membranes and of the cytoskeletal matrix of oxyntic cells was modified by the onset of HCl secretion. These results are in concert with the structural evidence of a redistribution of the membranes containing the proton pump (Smolka et al., 1983; Koenig, 1984; Fujimoto et al., 1986).

The secretory pole membrane fraction obtained from stimulated glands, mostly fragments of luminal processes, showed an Mg-ATPase activity at least threefold higher than that found in membranes of resting glands. Furthermore, an increase in K-ATPase activity, independent of valinomycin and a lower proportion of K-valinomycin–dependent ATPase, was found. Electron microscopy clearly showed that the luminal surface of the plasma membrane profiles isolated from stimulated glands was mainly oriented toward the suspension medium. K^+ activation in the absence of valinomycin might derive from this particular orientation of most membranes (Wallmark et al., 1980; Ljungstrom et al., 1984*a*, *b*) rather than from modifications in the permeability to KCl, as has been described in fractions isolated from stimulated mammalian parietal cells (Wolosin and Forte, 1981*a*, *b*, 1983; Im et al., 1984; Hirst and Forte, 1985).

Remarkable differences were found in the polypeptides that cosedimented in the purified light membrane fractions. The membrane fraction of resting glands, containing 70% units of K-NPPase, was enriched in a polypeptide of 95 kD that might correspond to the catalytic subunit of K-ATPase (Smolka et al., 1983; Ljungstrom et al., 1984). A change in the sedimentation pattern of polypeptides of 28, 43, 95, 200, and 250 kD was found when purified membrane fractions from resting and stimulated oxyntic cells were compared. These polypeptides might be associated with the K-NPPase–containing membranes. However, an artifact due to isolation procedures cannot be discarded. Since actin, myosin, and filamin have been related to the structural reorganization of oxyntic cell secretory pole (Vial and Garrido, 1976; Dabiké et al., 1986), further experiments are needed to elucidate this point. The striking increase of the 43-kD polypeptide in the fraction of stimulated glands agrees well with the presence of microfilaments inside the isolated membrane profiles.

Changes induced by the HCl secretory state on the specific activity of K-NPPase and Mg-ATPase might indicate the existence of a regulatory mechanism that acts on the transport functions of the oxyntic cell secretory membrane. Gastric K-NPPase activity can be regulated by an endogenous activator, associated as an extrinsic protein with gastric membranous vesicles (Ray, 1978; Ray and Nandi, 1983). Physiological HCl secretagogues activate the proton pump through second messengers such as Ca^{++} or cAMP, known to affect cytoskeletal functions (Rasmussen and Barret, 1984). On the other hand, it has been established that the structural reorganization starts before the onset of HCl secretion (Jiron et al., 1984). Drugs such as omeprazole, which dissociate the structural reorganization from HCl secretion, are direct inhibitors of ATPase (Helander and Sundell, 1984).

The inhibition of K-NPPase activity in HCl-secreting glands, in which stimulation of the proton pump must occur, agrees with the fact that the K-phosphatase component (measured as K-NPPase) is activated when the ATPase system is partially uncoupled. Several agents such as thimerosal (Forte et al., 1981*b*) or FITC (Jackson et al., 1983) reduce K-ATPase activity and enhance K-NPPase activity by interacting specifically with chemical groups in the microenvironment of the K-ATPase.

The biological function of the membrane Mg-ATPase is still unknown; it might either correspond to an expression of the K-ATPase system (Wallmark et al., 1980) or be a different enzyme. The main modification in the properties of isolated membrane fractions of mammalian parietal cells is a change in Cl^- conductance (Wolosin and Forte, 1981*a*, *b*, 1983; Ljungstrom et al., 1984). Even though the absolute depen-

dence on chloride for acid secretion has been established (Berglindh, 1984), the way in which oxyntic cells handle Cl^- is still unknown. It is accepted that the intracellular level of Cl^- required for HCl secretion is maintained by the active transport of Na/K at the basolateral membrane (Diamond and Machen, 1983). However, there is evidence of a Cl^- transport operating in the luminal plasma membrane in stimulated cells (Malinowska et al., 1983). On the other hand, according to the electrogenic theory for HCl secretion (Rehm et al., 1983), a pump for Cl^- acting in parallel with the proton pump must exist in the luminal membrane. A relevant issue is whether Mg-ATPase enhancement in stimulated glands would be related to activation of this putative Cl^- pump.

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