

## SECRETORY POTENTIAL AND IONIC TRANSPORT IN THE POSTERIOR SILK GLANDS OF *BOMBYX MORI*

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### SUMMARY

1. The concentrations of Na, Mg, P, S, Cl, K and Ca in the cytoplasm and lumen of the posterior silk gland cells of *Bombyx mori* were measured by X-ray microprobe analysis of freeze-dried thin sections. The basal and luminal membrane potentials of the gland cells were measured using microelectrode techniques. The input resistance of the luminal plasma membrane was simultaneously measured by injecting electric current *via* an intracellular microelectrode.

2. The basolateral membrane potential was  $-47 \pm 1.8$  mV (S.E.) ( $N = 46$ ), and the glands exhibited lumen-negative voltages of  $-6 \pm 0.1$  mV (S.E.) ( $N = 40$ ) in the normal state. Increasing the extracellular  $K^+$  concentration depolarized the basolateral membrane potential, whereas the membrane potential hyperpolarized when  $Cl^-$  concentration in the extracellular fluid was increased. There were no significant effects on the membrane potential when  $Na^+$ ,  $Mg^{2+}$  and  $Ca^{2+}$  concentrations in the extracellular fluid were changed.

3. The representative X-ray spectra showed high K and phosphorus peaks, and low Cl and Mg peaks in the cytoplasm of the normal posterior silk gland cells. The normal glandular lumen showed relatively high K, and low Cl, sulphur, Ca and Mg peaks. Quantitative microprobe values were, for the cytoplasm ( $mmol\ kg^{-1}$  wet mass,  $N = 30$ ) Na, 5; Mg, 14; phosphorus, 168; sulphur, 16; Cl, 12; K, 168; Ca, 0.5; and for the lumen ( $N = 10$ ) Na, 3; Mg, 25; phosphorus, 42; sulphur, 24; Cl, 38; K, 133; Ca, 9.4 in the normal glands.

4. The basal plasma membrane potential was hyperpolarized by 7 mV after stimulation with  $5 \times 10^{-5}$   $mol\ l^{-1}$  5-hydroxytryptamine (5-HT). Microprobe values for the cytoplasm were ( $mmol\ kg^{-1}$  wet mass,  $N = 15$ ) Na, 4; Mg, 13; phosphorus, 160; sulphur, 17; Cl, 8; K, 187; Ca, 0.6 in the stimulated glands. The cytoplasmic [K] increased after stimulation with 5-HT.

5. The basal membrane potential of the gland cells was depolarized by 3 mV after application of a juvenoid, methoprene ( $10^{-5}$   $mol\ l^{-1}$ ). X-ray microprobe values for the cytoplasm were ( $mmol\ kg^{-1}$  wet mass,  $N = 15$ ) Na, 7; Mg, 11; P, 170; S, 14; Cl, 23; K, 130; Ca, 3.4 in the treated glands. The cytoplasmic [Ca] and [Cl] increased, while the [K] decreased with methoprene stimulation.

6. The luminal membrane potential of the gland cells was depolarized by 8 mV and a simultaneous decrease of luminal membrane resistance was apparent after stimulation with an anti-microfilament reagent, cytochalasin D ( $2 \times 10^{-6}$   $mol\ l^{-1}$ ).

Key words: secretory potential, ionic transport, silk gland.

X-ray microprobe values for the cytoplasm became ( $\text{mmol kg}^{-1}$  wet mass,  $N = 15$ ) Na, 10; Mg, 28; P, 194; S, 22; Cl, 24; K, 148; Ca, 1.2; and for the lumen ( $N = 15$ ) Na, 14; Mg, 13; phosphorus, 31; sulphur, 30; Cl, 92; K, 122; Ca, 1.1 in the stimulated glands. The cytoplasmic [Ca] and [Cl] increased and [K] decreased, whereas the luminal [Cl] and [sulphur] increased and [Ca] and [Mg] decreased after cytochalasin D stimulation.

7. The reaction products of adenosine triphosphatase activity were found on the luminal and lateral plasma membranes of the posterior silk gland cells.

8. The possible routes of ion transport into the lumen are discussed.

#### INTRODUCTION

The relationship between the intracellular ionic environments and the secretion of exocrine glands has been extensively studied. Calcium is known to have a central role in the secretory process, and physiological stimulants leading to enzyme and fluid secretion exert marked changes on the bioelectrical properties of the cell membrane of exocrine glands (Kanno, 1972; Matthews, Petersen & Williams, 1973; Berridge, Lindley & Prince, 1975; Petersen, 1980; Ginsborg & House, 1980).

It has also been suggested that microtubules and/or microfilaments play some role in the secretion process of various organs such as the pancreas (Lacy, Howell, Young & Fink, 1968; Baudin, Stock, Vincent & Grenier, 1975), salivary gland (Rossignol, Herman & Kerger, 1972; Nakagaki, Goto, Sasaki & Imai, 1978) and adrenal medulla (Douglas & Sorimachi, 1972; Poisner & Banerjee, 1971).

The posterior silk gland of the silkworm is a tubular epithelium which is large and differentiated for the synthesis of the silk protein fibroin, and it secretes a protein-rich fluid into the glandular lumen. These large cells have a radial microtubular system and a circular microtubule-microfilament system (Fig. 5A,B). These systems are concerned with the intracellular transport and secretion of fibroin (Sasaki & Tashiro, 1976). We reported that, in the presence of colchicine or vinblastine, the secretion of fibroin was suppressed and that there was a marked accumulation of the secretory granules of fibroin in the Golgi regions. The secretion was accelerated and extensive invagination of the luminal surface was observed in the presence of cytochalasin B or D (Sasaki, Nakajima, Fujii-Kuriyama & Tashiro, 1981).

Some investigators reported that a suitable dose of juvenile hormone (methoprene) stimulated the RNA and fibroin synthesis of the posterior silk gland cells and brought about an increase in weight of the cocoon shell, due to an enhancement of fibroin secretion into the lumen (Henrick *et al.* 1975; Akai, Kiguchi & Mori, 1971, 1973).

There is general agreement that the intracellular transport of ions and water is driven by active ion transport systems on the luminal plasma membranes of insect exocrine gland cells. This transport is enhanced by exposure to the neurotransmitter serotonin (5-hydroxytryptamine) (Gupta, Berridge, Hall & Moreton, 1978).

We investigated properties of the secreting system in the posterior silk glands of *Bombyx mori*, using electrophysiological and electron microprobe techniques. Special reference was made to changes in the basolateral and luminal plasma

membrane potentials, and cytoplasmic and luminal ion concentrations in the secretory stimulated states.

## MATERIALS AND METHODS

### *Materials*

The strain of silkworm used is a hybrid of Shunrei and Shogetsu. The posterior silk gland cells of the silkworm, *Bombyx mori*, are very large hexagonal cells, each of which extends for as much as half the circumference of the gland, the cell dimensions being (approximately)  $1.3 \text{ mm} \times 1.6 \text{ mm} \times 0.17 \text{ mm}$  at the maturation stage of the fifth instar. Their function is to synthesize and secrete a large amount of fibroin into the lumen, the diameter of which is about  $0.7 \text{ mm}$ .

### *Microelectrode measurements*

The posterior silk glands in the later stages of the fifth larval instar are about  $15 \text{ cm}$  long. They can be removed from the abdomen of the silkworms, and incubated in Wyatt's (1956) insect tissue culture medium, or in artificial haemolymph (pH 6.8) which contains ( $\text{mmol l}^{-1}$ )  $\text{K}^+$ , 30;  $\text{Na}^+$ , 11;  $\text{Ca}^{2+}$ , 12;  $\text{Mg}^{2+}$ , 23;  $\text{Cl}^-$ , 79;  $\text{NaHCO}_3$ , 4;  $\text{NaH}_2\text{PO}_4$ , 7. The osmolarity was adjusted to  $300 \text{ mosmol l}^{-1}$  by adding mannitol, and the medium was gassed continuously with 95%  $\text{O}_2$  and 5%  $\text{CO}_2$ , maintained at  $37^\circ\text{C}$ . The  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  concentrations were altered by various salts:  $\text{Na}^+$  and  $\text{K}^+$  were replaced with Tris or tetraethylammonium and  $\text{Cl}^-$  was replaced with sulphate. The lumen of the posterior silk glands was perfused with a modified solution and the lumen was separated from the bath compartment. The modified luminal solution used mimicked the luminal contents, as estimated from the X-ray microanalysis and/or flame photometry. The concentrations were ( $\text{mmol l}^{-1}$ ):  $\text{Na}^+$ , 10;  $\text{Mg}^{2+}$ , 25;  $\text{Cl}^-$ , 42;  $\text{K}^+$ , 84;  $\text{Ca}^{2+}$ , 9.4.

Glass microelectrodes filled with  $3 \text{ mol l}^{-1}$  KCl, with a tip resistance between 5 and  $20 \text{ M}\Omega$ , were used. The microelectrode was connected to a differential type amplifier (Nihon Kohden MEZ-7101) allowing for current injection and simultaneous measurements of membrane potential and input resistance. The potential and resistance changes were recorded using an oscilloscope and a pen recorder. The microelectrode was advanced into the cell and further into the lumen, then retracted into the cell and further out into the extracellular space. Sometimes the reference was also taken into the lumen.

Serotonin (5-HT) (Merck) was added to the bath solution at a final concentration of  $5 \times 10^{-5} \text{ mol l}^{-1}$ . Methoprene (Otsuka Chemical Co., Japan) was dissolved in ethanol ( $1.25 \text{ mg ml}^{-1}$ ) and added to the bath solution at a final concentration of  $10^{-5} \text{ mol l}^{-1}$ . Cytochalasin D (Sigma) was dissolved in dimethyl sulphoxide (DMSO) ( $1 \text{ mg ml}^{-1}$ ) and added to the bath solution at a final concentration of  $2 \times 10^{-6} \text{ mol l}^{-1}$ . Addition of ethanol or DMSO at the same concentrations used in the experiments did not produce any significant change in the electrical and ionic properties of the silk glands.

We recorded changes in the membrane potential of the basolateral and luminal plasma membranes, the resistance of the luminal membrane and the transcellular potential differences.

#### *Elements in whole tissues*

The  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  concentrations in the whole glands, cells and lumen of the posterior silk glands were determined by flame photometry (Eppendorf) and by chloridometer (Buchler, Inc.), respectively.

#### *X-ray microanalysis*

For electron probe X-ray microanalysis, the posterior silk glands in the resting and treated (1–30 min after the additions of reagents) states were put on copper specimen holders and rapidly frozen by pressing them against the wall of a copper block which had been precooled in liquid nitrogen. 100–200 nm thick cryosections were cut on the frozen thin sectioner (FTS) of a Poter Blum MT-2 ultramicrotome maintained at  $-150^\circ\text{C}$ , and the sections were mounted on gold or titanium grids and transferred to an FTS freeze-drier (FTS Systems, Inc., Stone Ridge, NY, USA). The preparations were dried at  $10^{-3}$ – $10^{-4}$  mmHg overnight, followed by carbon coating. The X-ray microanalysis was done using a Hitachi H-500 electron microscope interfaced with a KeveX Si(Li) detector and 5100 multi-channel analyser. The microanalyser was operated at 75 kV. A probe current of  $10^{-9}$ – $10^{-10}$  A was used and the analysis was usually for 100 s. For the estimation of local dry mass fractions, an analysis was made of the frozen sections. The grids with frozen sections were set in the cooling specimen holder of a Hitachi H-500 electron microscope (H 5001 C) in the ultramicrotome and the X-ray microanalysis was carried out immediately (Sasaki, Nakagaki, Mori & Imai, 1983). We used only the spectra in which the gold peak was low. The X-ray energy spectra and further data processing to obtain the final concentration values were performed using an on-line computer system (Hitachi MB-MA 16003, CPU: 8088, MS-DOS). The special utility programs included some statistical analysis. Details of the procedures have been reported elsewhere (Nakagaki, Sasaki, Shiguma & Imai, 1984).

#### *Cytochemical demonstration of ATPase*

The cytochemical localization of ATPase was carried out according to the methods of Wachstein & Meisel (1957) and Marchesi & Palade (1967), as reported previously (Nakagaki *et al.* 1978).

## RESULTS

#### *Membrane potentials of the posterior silk gland cells*

Fig. 1 shows a representative intracellular *in vitro* recording of the membrane and transepithelial potential, with reference to the extracellular bath solution. The average basolateral membrane potential was  $-47 \pm 1.8$  mV (S.E.) ( $N = 46$ ), and the

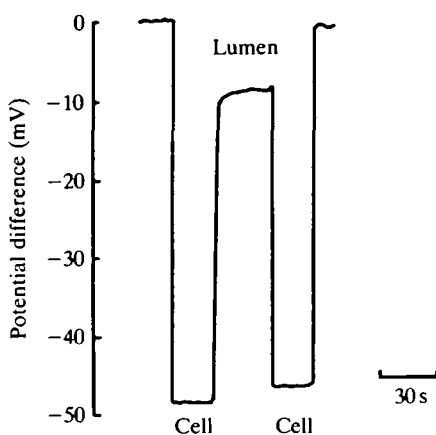


Fig. 1. Profiles of transmembrane and transepithelial potential differences across the posterior silk gland cells.

average transcellular potential difference was  $6 \pm 0.1$  mV (S.E.) ( $N = 40$ ). The glands exhibited lumen-negative voltages of  $-6$  mV.

The effects of various extracellular ions on the basolateral membrane potential were investigated (Fig. 2). Increasing extracellular  $[K^+]$  depolarized the membrane and the slope was 10 mV for a ten-fold change in  $[K^+]$ . The membrane hyperpolarized when  $[Cl^-]$  in the extracellular fluid was increased. The slope of the line obtained was 30 mV for a ten-fold change in  $[Cl^-]$ . There were no significant effects on the membrane potential when  $Na^+$ ,  $Mg^{2+}$  and  $Ca^{2+}$  concentrations in the extracellular fluid were changed (data not shown). The lines of basal membrane potentials at different concentrations of potassium and chloride in the extracellular solution lay between the Nernst plots for potassium and chloride, estimated from the cytoplasmic  $K^+$  and  $Cl^-$  concentrations given in Table 1 and those found in the extracellular fluid (Fig. 2).

#### *The effects of 5-HT, methoprene and cytochalasin on potential responses*

Serotonin acts as a secretagogue and enhances ion secretion in insect gland cells (Gupta *et al.* 1978). We found that the basal membrane potential of the posterior silk gland cells was hyperpolarized to 7 mV by treatment with 5-HT ( $5 \times 10^{-5}$  mmol  $l^{-1}$ ) (Fig. 3A; Table 1).

A suitable dose of juvenoid stimulates fibroin synthesis in the posterior silk gland cells and the cocoon shell increases in mass (Akai *et al.* 1971). In our experiments, the basal membrane potential was depolarized by application of a juvenoid, methoprene ( $10^{-5}$  mmol  $l^{-1}$ ) (Fig. 3B). The mean change in basolateral membrane potential was 5 mV following the application of methoprene (Table 1).

Cytochalasin D, an anti-microfilament reagent, effectively facilitated the secretion of fibroin and caused extensive invagination of the luminal surface. These events

were attributed to the serial exocytosis of fibroin globules in the posterior silk gland cells (Sasaki *et al.* 1981). Addition of cytochalasin D ( $2 \times 10^{-6}$  mmol l<sup>-1</sup>) to the bath solution caused a depolarization of 8.0 mV in the luminal membrane, and there was a simultaneous decrease in membrane resistance (Fig. 4; Table 2). The mean resting input resistance was  $970 \pm 210$  k $\Omega$  ( $N = 10$ ) and the mean input resistance of glands treated with cytochalasin D was  $360 \pm 140$  k $\Omega$  ( $N = 7$ ) across the luminal membrane.

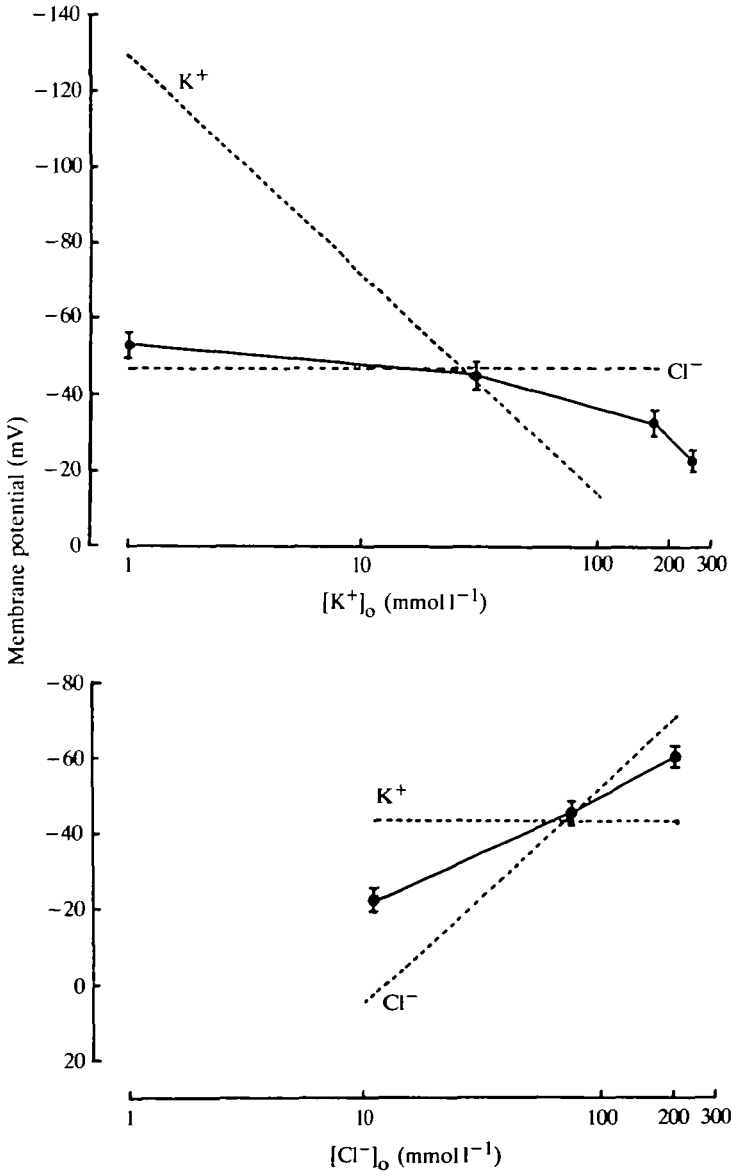


Fig. 2. Dependence of the posterior silk gland cell membrane potential on external K<sup>+</sup> (A) and Cl<sup>-</sup> (B) concentrations. The dashed lines indicate the K<sup>+</sup> and Cl<sup>-</sup> equilibrium potentials calculated from the Nernst equation.

Table 1. Effects of serotonin (5-HT) and a juvenoid (methoprene) on the basal membrane potential (mean  $\pm$  S.E.; mV) and cytoplasmic elemental concentration (mean  $\pm$  S.E.; mmol kg<sup>-1</sup> wet mass) of silk gland cells measured by electron probe X-ray microanalysis of freeze-dried sections

	Control	5-HT	Methoprene
Basal membrane potential	-47 $\pm$ 1.8	-54 $\pm$ 1.5*	-42 $\pm$ 0.9*
N	46	32	25
Element			
Cytoplasm			
Na	5 $\pm$ 0.5	4 $\pm$ 0.8	7 $\pm$ 1.3
Mg	14 $\pm$ 1.5	13 $\pm$ 2	11 $\pm$ 2.5
P	168 $\pm$ 6.8	160 $\pm$ 8	170 $\pm$ 3.4
S	16 $\pm$ 0.6	17 $\pm$ 1.3	14 $\pm$ 0.8
Cl	12 $\pm$ 1.6	8 $\pm$ 1.3	23 $\pm$ 1.5*
K	168 $\pm$ 3.3	187 $\pm$ 5.4*	130 $\pm$ 3.6*
Ca	0.5 $\pm$ 0.1	0.6 $\pm$ 0.1	3.4 $\pm$ 0.5*
N	30	15	15

\* $P < 0.005$ , comparing control and treated cells.

The basal membrane potential is depolarized after exposure to cytochalasin D (Sasaki & Nakagaki, 1979).

#### Cytochemistry of ATPase

Electron microscopy reveals the radial microtubule and circular microtubule-microfilament systems in perinuclear and laminal cytoplasm, respectively

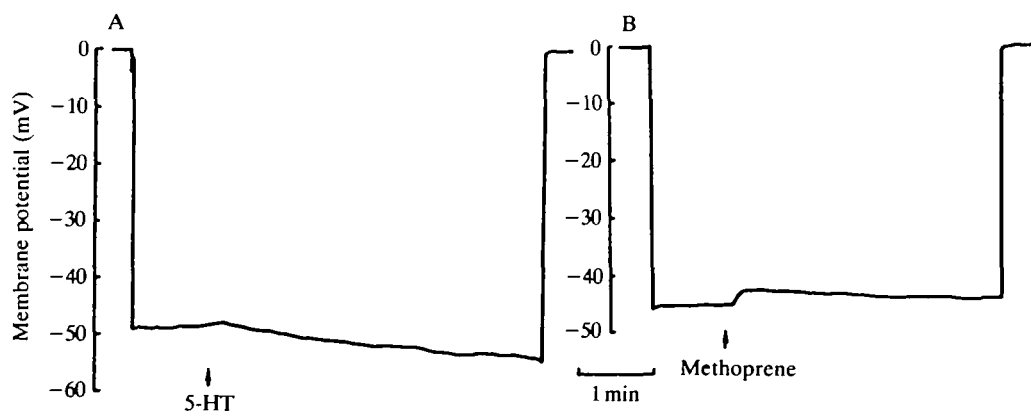


Fig. 3. Basal membrane potential changes induced by serotonin (5-HT) ( $1 \times 10^{-5}$  mol l<sup>-1</sup>) (A) and a juvenoid (methoprene) ( $1 \times 10^{-5}$  mol l<sup>-1</sup>) (B) in gland cells. At the arrows, one drop of the secretagogue was added to the bath solution. Addition of ethanol only (used as a solute for methoprene) produced no significant changes.

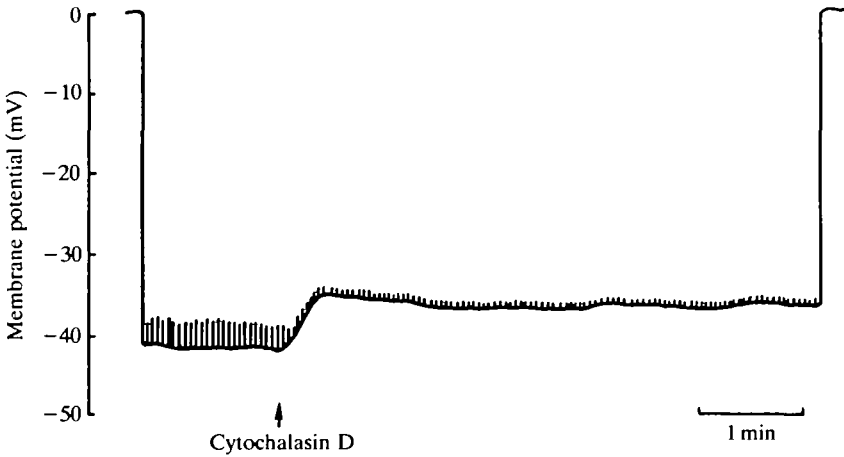


Fig. 4. Luminal membrane potential and resistance changes induced by cytochalasin D in the posterior silk gland cells. At the arrow cytochalasin D ( $1 \times 10^{-5} \text{ mol l}^{-1}$ ) was added to the bath solution. Current pulses (5 nA) were injected through the recording microelectrode. The addition of DMSO (the solute for cytochalasin D) produced no significant changes.

(Fig. 5A,B), and the extensively developed basal infoldings of the plasma membranes of the posterior silk gland cells (Fig. 6). ATPase reaction products were not present on these membranes. Golgi apparatus, fibroin globules and endoplasmic reticulum were present in the cytoplasm (Fig. 6A). The reaction products of ATPase activity were found on the lateral and luminal plasma membranes and on the membrane of luminal microvilli (Fig. 6B).

#### *Microprobe analysis*

Scanning electron micrographs of freshly freeze-dried thin sections revealed nuclei, nucleoli and cytoplasm (Fig. 5C).

Representative X-ray spectra over the cytoplasm and lumen of dehydrated sections are shown in Figs 7 and 8. There are high K and P peaks, and low Cl and Mg peaks over the basal cytoplasm of the normal glands. The apical cytoplasm shows higher Cl and lower P peaks as compared with the basal cytoplasm, and fibroin globules and endoplasmic reticulum contain Ca (spectra not shown). The peripheral glandular lumen shows relatively high K, and Cl, S, Ca and Mg peaks. The concentrations of these elements in the cytoplasm and lumen are shown in Tables 1 and 2. The K concentration in the cytoplasm was over  $168 \text{ mmol kg}^{-1}$  wet mass, which is higher than in other exocrine cells such as blowfly salivary gland (Gupta *et al.* 1978; Gupta & Hall, 1983) and mammalian salivary gland and pancreas (Sasaki *et al.* 1983; Nakagaki *et al.* 1984). The bathing medium and the haemolymph *in vivo* have  $30 \text{ mmol l}^{-1} \text{ K}^+$  as compared with  $10 \text{ mmol l}^{-1}$  in the blowfly and only about  $3 \text{ mmol l}^{-1}$  in mammalian systems. The intracellular and extracellular  $\text{K}^+$  concentrations in the silk glands measured by ion-selective microelectrodes were  $177 \pm 21.5$



and  $30 \pm 1.2 \text{ mmol kg}^{-1} \text{ H}_2\text{O}$ ,  $N = 32$ , respectively (Sasaki *et al.* 1977). These values estimated from the microprobe study and the microelectrode technique suggest that part of the intracellular potassium exists in a bound form. Phosphorus was present in the cytoplasm at a relatively high concentration ( $168\text{--}177 \text{ mmol kg}^{-1}$ ), similar to that in canine pancreatic acinar cells (Nakagaki *et al.* 1984). The cytoplasm contained a large quantity of endoplasmic reticulum membrane, and membrane-bound ribosomes which synthesize exportable protein, such as fibroin, in the silk gland, and amylase, lipase etc. in the pancreas.

The glandular lumen showed higher Mg, S, Cl and Ca levels and lower P and K levels than were seen in the cytoplasm; the higher Mg, S and Ca levels can be attributed to exocytosis of fibroin globules into the lumen (Table 2). The phosphorus content of the lumen may have included some in organic form, because the luminal value was relatively high. There is a deficiency of anions in the lumen contents (Table 2), perhaps due to a loss of elements such as chloride from luminal compartments in the tissue during freeze-drying.

Table 2. *Effects of cytochalasin D on the luminal membrane potential (mean  $\pm$  S.E.; mV) and elemental concentrations (mean  $\pm$  S.E.; mmol kg<sup>-1</sup> wet mass) of silk gland cells measured by electron probe X-ray microanalysis of freeze-dried sections*

	Control	Cytochalasin D
Luminal membrane potential	$-41 \pm 0.9$	$-33 \pm 1.4^*$
N	36	20
Element		
Cytoplasm		
Na	$5 \pm 0.6$	$10 \pm 1.8$
Mg	$24 \pm 1$	$28 \pm 3$
P	$177 \pm 6.6$	$194 \pm 12$
S	$20 \pm 0.8$	$22 \pm 0.6$
Cl	$15 \pm 1.8$	$24 \pm 2.3^*$
K	$175 \pm 8.4$	$148 \pm 5.4^*$
Ca	$0.4 \pm 0.02$	$1.2 \pm 0.2^*$
N	25	15
Lumen		
Na	$3 \pm 0.4$	$14 \pm 2.1$
Mg	$25 \pm 0.5$	$13 \pm 2.3^*$
P	$42 \pm 3.2$	$31 \pm 3.1$
S	$24 \pm 2$	$30 \pm 1.8^*$
Cl	$38 \pm 2.7$	$92 \pm 8.5^*$
K	$133 \pm 9.2$	$122 \pm 6.7$
Ca	$9.4 \pm 1.2$	$1.1 \pm 0.7^*$
N	19	15

\* $P < 0.005$ , comparing control and treated glands.

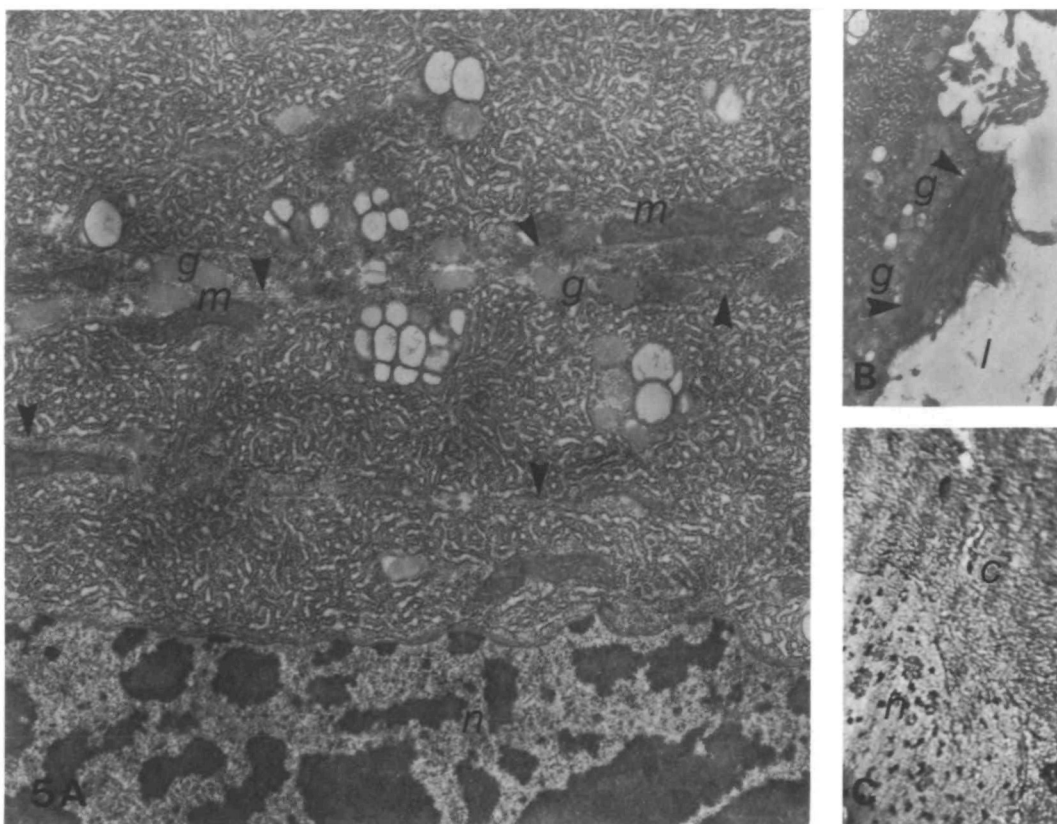


Fig. 5. Conventional electron micrographs of perinuclear cytoplasm (A) and luminal cytoplasm (B) of posterior silk gland cells, and scanning transmission electron micrograph of a freeze-dried thin section of posterior silk gland cells (C). (A) Microtubules run from basal to luminal membrane in the cytoplasm, and mitochondria (*m*) and fibroin globules (*g*) can be seen in close proximity to the microtubules (arrowheads); *n*, nucleus.  $\times 13\,500$ . (B) A circular microtubule system (arrowheads) can be seen in the luminal cytoplasm, and numerous fibroin globules (*g*) are visible; *l*, lumen.  $\times 6500$ . (C) Scanning electron micrographs of freshly frozen, dried, thin sections of a posterior silk gland cell showing nucleus (*n*), nucleolus and cytoplasm (*c*).  $\times 4500$ .

In the X-ray spectra of the cytoplasm, the K concentration increased when the gland was treated with serotonin (5-HT) and, following treatment with methoprene, the concentration of cytoplasmic Ca increased slightly whereas the K concentration decreased (Fig. 7; Table 1).

After cytochalasin D treatment, [Cl] and [Ca] in the cytoplasm increased, and [Cl] in the lumen increased (Fig. 8; Table 2). These results suggest that the transcellular Cl<sup>-</sup> flow from the basal extracellular space to the lumen of the posterior silk gland occurs as a result of the secretory state of the gland cells.

The dry mass fraction was estimated to be 25% in the cytoplasm and 34% in the glandular lumen, using frozen hydrated and dehydrated sections of the posterior silk

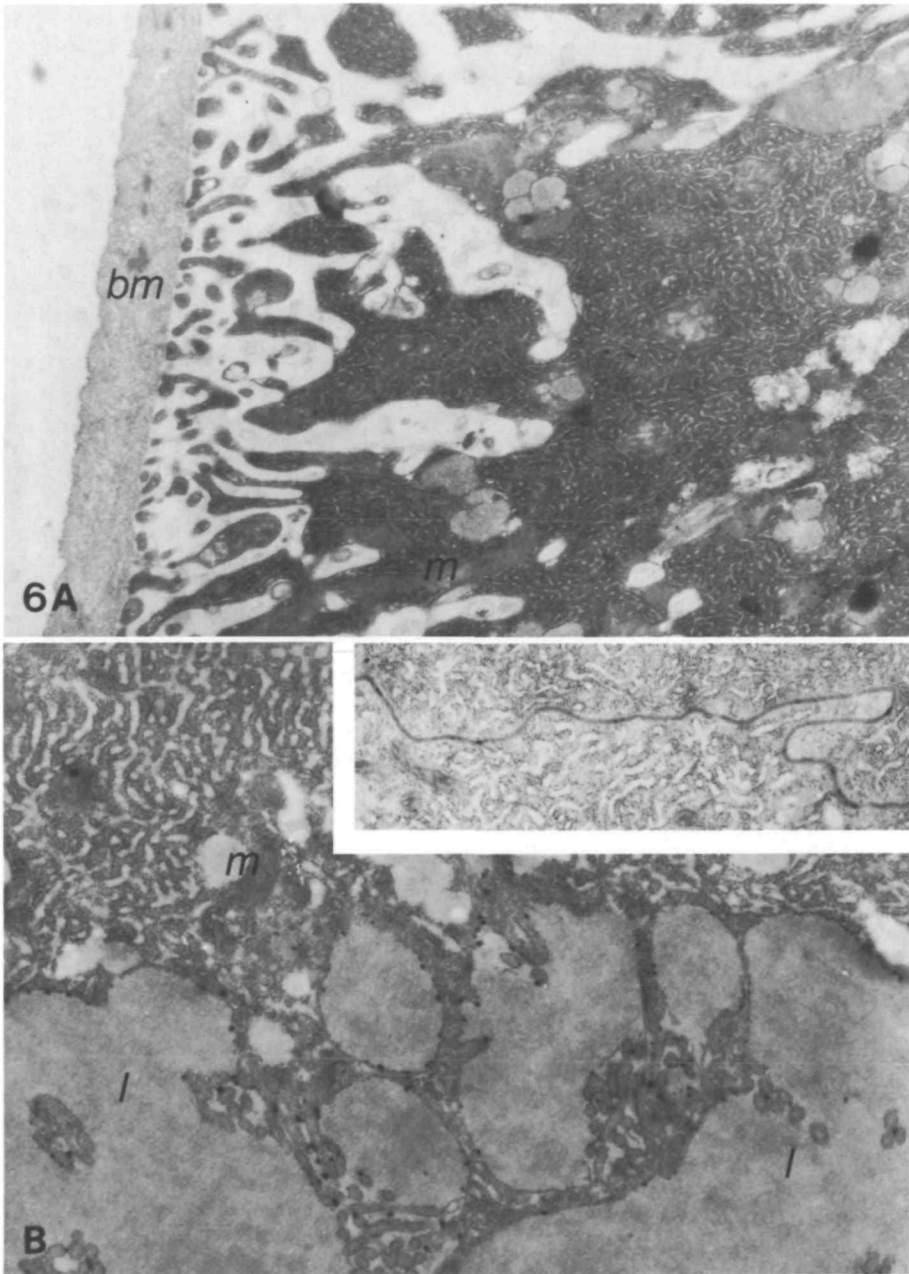


Fig. 6. Electron micrographs of the basal (A) and luminal (B) portion of the posterior silk gland cells stained by the adenosine triphosphatase cytochemical reaction. Reaction products of the ATPase activity are found on the membrane of microvilli in the luminal portion of the cell and can also be seen on the lateral membrane of the cells (B inset). There are no reaction products on the basal plasma membrane (A). *bm*, basement membrane; *m*, mitochondria; *l*, lumen. A  $\times 17\,000$ ; B  $\times 17\,000$ ; inset,  $\times 11\,000$ .

glands, in the resting state. The dry mass fraction of the 5-HT-treated cytoplasm increased slightly, and this increase was less than that seen in the blowfly salivary glands (Gupta & Hall, 1981). The dry mass fractions of cytoplasm treated with methoprene and cytochalasin and of lumen treated with cytochalasin remained unchanged.

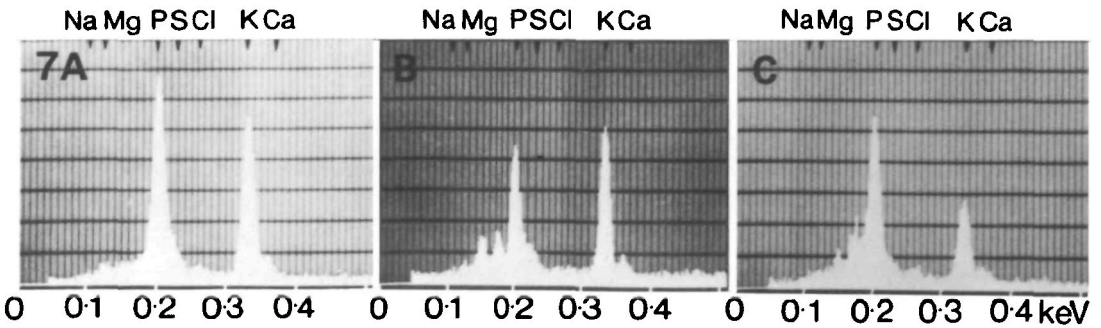


Fig. 7. Energy dispersive X-ray spectra from cytoplasm of normal (A), and 5-HT- (B) and methoprene- (C) treated gland cells. Non-fixed, freeze-dried thin sections were obtained from freshly frozen posterior silk glands. Note the higher K peak in the spectrum obtained from the gland treated with 5-HT and the lower K peak in the spectrum obtained from the methoprene-treated gland.

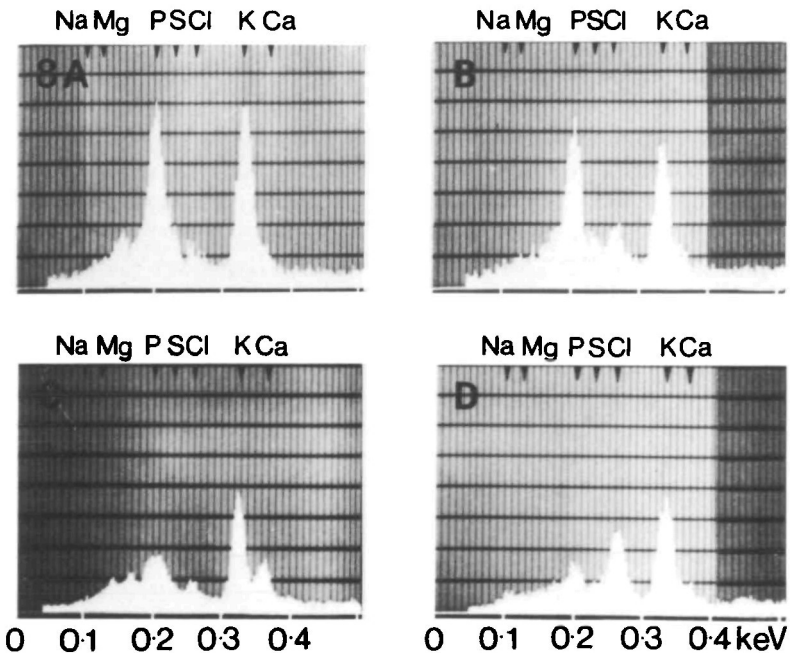


Fig. 8. Energy dispersive X-ray spectra of the cytoplasm (A,B) and glandular lumen (C,D) of freshly freeze-dried thin sections from normal (A,C) and cytochalasin-treated (B,D) gland cells. Note the higher Cl peaks in the spectra of cytoplasm and lumen treated with cytochalasin D.

*Measurements of elements in whole tissues*

Using the flame photometer and a chloridometer, we measured the  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  concentrations in the resting posterior silk glands. Levels in the whole glands were  $13 \pm 2.1$ ,  $108 \pm 6.5$  and  $20 \pm 3.5$  ( $\pm$ S.D.)  $\text{mmol kg}^{-1}$  wet tissue,  $N = 14$ , respectively. The lumen is partitioned into regions occupied by newly discharged fibroin, a thin layer of fibroin, and a columnar fibroin filled with material of moderate density (Sasaki & Tashiro, 1976). The ion concentrations in the part of the lumen filled with columnar fibroin were:  $\text{Na}^+$ ,  $16 \pm 3.4$ ;  $\text{K}^+$ ,  $35 \pm 5.7$ ;  $\text{Cl}^-$ ,  $45 \pm 5.3$   $\text{mmol kg}^{-1}$ . The  $\text{K}^+$  value given here is very low compared with the data for the lumen measured by X-ray microanalysis. This large discrepancy indicates that the microprobe analysis measured the peripheral parts of the lumen close to the apical cytoplasm: as a result, some of the microvilli were included and/or this layer may have a large  $\text{K}^+$  concentration gradient. The concentrations in the cellular part, which remains after removal of columnar fibroin from the gland, were  $\text{Na}^+$ ,  $6 \pm 1.4$ ;  $\text{K}^+$ ,  $163 \pm 5.8$ ;  $\text{Cl}^-$ ,  $18 \pm 2.3$  ( $\pm$ S.D.)  $\text{mmol kg}^{-1}$  wet tissue,  $N = 14$ .

## DISCUSSION

The posterior silk gland consists of very large cells highly specialized for synthesis, intracellular transport and secretion of protein (Sasaki & Tashiro, 1976; Sasaki *et al.* 1981). There are many similarities between protein production in the silk gland and in the pancreatic exocrine gland. Both preparations have all the structural features of a typical protein-secreting gland (Palade, 1975). The posterior silk gland has a large lumen, which facilitates direct measurements of membrane potentials and ionic concentrations in the various compartments, such as cytoplasm and lumen, during secretory states. We investigated the mechanism of secretion of the posterior silk gland, using electron probe X-ray microanalysis, coupled with studies using microelectrode techniques.

Intracellular and transcellular recordings from the posterior silk gland cells in the normal state suggest that the plasma membrane of the gland cells is permeable to  $\text{K}^+$  and  $\text{Cl}^-$ , and that an anion such as  $\text{Cl}^-$  is transported passively and/or secondarily actively from the basal extracellular space to the gland lumen, in normal secretory states. The presence of adenosine triphosphatase activity, which is located on the luminal plasma membrane, also suggests that the posterior silk gland cells may have a cation pump, such as  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase and/or  $\text{H}^+$ ,  $\text{K}^+$ -ATPase, on the luminal surface, as the extracellular  $[\text{Na}^+]$  was low and the luminal pH was shown to be acidic using ion-selective microelectrodes: cytoplasm,  $\text{pH } 7.1 \pm 0.09$  ( $\pm$ S.D.); lumen,  $\text{pH } 6.2 \pm 0.1$  ( $\pm$ S.D.)  $N = 5$ . This cation pump would create the driving force for secondary active  $\text{Cl}^-$  transport. The  $\text{K}^+$  channel on the luminal membrane supplies  $\text{K}^+$  for this pump. Gupta & Hall (1983) proposed that in cockroach salivary glands, a cation pump, such as  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase, is located on the luminal plasma membrane and supplies the driving force for intracellular  $\text{Cl}^-$  transport from the serosal portion to the lumen of the acinar P-cells. However, the ionic composition in the luminal contents of posterior silk gland is similar to that of the goblet cavity in the

$K^+$ -secreting cells of the larval midgut in *Manduca sexta* (Dow, Gupta, Hall & Harvey, 1984; Gupta, Dow, Hall & Harvey, 1985). The evidence suggests that there may be an electrogenic  $K^+$  pump on the apical membrane and a conventional  $Na^+, K^+$ -pump on the basolateral plasma membrane. Greger, Schlatter & Lang (1983) proposed that in the thick ascending limbs of Henle's loop, the intracellular  $Cl^-$  transport of tubular cells could be explained by a secondary active process and that the  $Na^+, K^+$ -ATPase located on the peritubular plasma membrane would supply the driving force for  $Cl^-$  transport. Some workers, studying parietal cells of the rat or rabbit gastric mucosa, have found that  $H^+, K^+$ -ATPase, which is located on the tubulovesicular membrane and/or plasma membrane of microvilli of the secretory canaliculus, could provide the driving force for  $Cl^-$  transport into the lumen (Rabon *et al.* 1983). Although the parietal cells of the vertebrate stomach secrete isotonic HCl at pH 2.0, the gastric mucosa also generates a potential difference that is lumen-negative, as is the case in the posterior silk gland. The membranes of tubulovesicles surrounding the secretory canaliculi of gastric parietal cells have  $H^+, K^+$ -ATPase,  $K^+$  and  $Cl^-$  channels. It is thought that stimulation of these channels opens and activates the pump, and that  $Ca^{2+}$  is simultaneously released from an intracellular store, such as the tubulovesicular structures and/or the luminal membrane of the secretory canaliculi. Finally, this  $Ca^{2+}$  release enhances the exocytosis of the tubulovesicles into the canaliculus lumen, a process in which the cytoskeletal system is involved (Tsunoda & Mizuno, 1985).

In our study, which used 5-HT as the stimulus, the basal membrane was hyperpolarized and the intracellular  $K^+$  concentration was increased. We attribute these events to increases in the  $K^+$  permeability of the basal cell membrane and in the active transport of  $K^+$ , following stimulation of the  $K^+$ -pump on the apical membrane and the  $Na^+/K^+$  exchange pump on the basal cell membrane. This cation pump may be present in other epithelia, such as *Calliphora* salivary glands and Malpighian tubules (Berridge *et al.* 1975; Gupta, Hall, Maddrell & Moreton, 1976).

The basal membrane potential of the posterior silk gland cells became depolarized in the presence of the juvenile hormone, methoprene. This agent effectively increases synthesis and secretion of fibroin, resulting in the production of large cocoons at the final stage of the fifth instar. There are similarities between the chemical formula of methoprene and of unsaturated fatty acids (Henrick *et al.* 1975); this hormone may act on the gland cells in a manner similar to that of some unsaturated fatty acids such as arachidonic acid and/or some prostaglandins. Methoprene may affect the exocrine gland cells by acting as a transmitter and/or a peptide hormone, as do acetylcholine and gastrin-CCK-PZ. In the present microprobe study using methoprene, we observed an influx of  $Ca^{2+}$ , an efflux of  $K^+$ , and an increase in  $Cl^-$  transport into the lumen in silk gland cells. The  $Ca^{2+}$  influx and the  $Cl^-$  efflux *via* apical membranes explain the depolarization of the plasma membrane and the lumen-negative transepithelial potential. The rise in intracellular  $Ca^{2+}$  concentration also enhances the exocytotic mechanism of the secretory granules of fibroin in the luminal plasma membrane, which is carried out by the microtubule-microfilament systems of the cells. These data also suggest that the  $Ca^{2+}$ -regulated

$\text{Cl}^-$  transport into the lumen is stimulated by treatment with the juvenile hormone, methoprene, following which the voltage-dependent and  $\text{Ca}^{2+}$ -regulated  $\text{K}^+$  channels in the basolateral plasma membranes are opened (Maruyama, Gallacher & Petersen, 1983; Petersen & Maruyama, 1984; Trautman & Marty, 1984; Marty, Tan & Trautman, 1984).

Cytochalasin B or D accelerates exocytosis of the secretory granules of fibroin into the luminal portion of the gland cells and enhances the secretion of fibroin into the lumen of the posterior silk gland (Sasaki *et al.* 1981). In this and previous studies, intracellular recordings of the luminal plasma membrane potential of the gland cells showed a depolarization and the input resistance of the membrane decreased when the gland was treated with cytochalasin B or D (Sasaki & Nakagaki, 1979). In the present study, we have shown that the intracellular  $\text{Ca}^{2+}$  concentration increased and that  $\text{Cl}^-$  transport into the lumen occurred in gland cells treated with cytochalasin D. The increase in cytoplasmic  $\text{Ca}^{2+}$  concentration may be important in the regulation by  $\text{Ca}^{2+}$  of transcellular  $\text{Cl}^-$  transport. Moreover, the increase in cytoplasmic  $[\text{Ca}^{2+}]$  and the decrease in luminal  $[\text{Ca}^{2+}]$  indicate that  $\text{Ca}^{2+}$  is released from fibroin globules, which serve as an intracellular store for  $\text{Ca}^{2+}$ . Further, this  $\text{Cl}^-$  transport is thought to be a secondary active process, the driving force for which is derived from  $\text{Na}^+, \text{K}^+$ -ATPase located on the lateral plasma membrane or a  $\text{K}^+$  pump such as  $\text{H}^+, \text{K}^+$ -ATPase, and an electrogenic pump on the luminal plasma membrane, as in the case of salivary glands and gastric mucosa (Berridge *et al.* 1975; Gupta & Hall, 1983; Rabon *et al.* 1983). This  $\text{Cl}^-$  transport system would explain our electrophysiological data and may be regulated by cytoplasmic  $\text{Ca}^{2+}$ , derived mainly from intracellular  $\text{Ca}^{2+}$  stores such as secretory granules and/or endoplasmic reticulum (Sasaki *et al.* 1983; Streb, Irvine, Berridge & Shultz, 1983; Nakagaki *et al.* 1984; Berridge & Irvine, 1984). This  $\text{Ca}^{2+}$  would enhance exocytotic mechanisms, and also affect the microtubule-microfilament systems in the luminal portion of the gland cells.

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