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CHANGES IN ELEMENTAL CONCENTRATIONS OF RAT PAROTID ACINAR CELLS FOLLOWING PILOCARPINE STIMULATION

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SUMMARY

1. Quantitative electron microprobe analysis was used to measure elemental dry weight concentrations in cytoplasm, secretory granules and nuclei of resting and pilocarpine-stimulated rat parotid gland acinar cells.

2. Secretory granules in resting cells had lower concentrations of Na, Mg, P, Cl and K, and higher concentrations of S and Ca than cytoplasm or nuclei. Nuclei in resting cells had lower S and higher K concentrations than cytoplasm.

3. Three major pilocarpine-related changes were found: (i) cytoplasmic dry weight concentrations of Na and Ca increased and the concentration of K decreased, (ii) the nuclear concentration of Na increased while that of K decreased and (iii) the concentrations of Na and Cl increased in secretory granules.

4. These results indicate that the nuclear, and cytoplasmic compartments have different mechanisms for regulating their elemental concentrations relative to the secretory granules.

5. The present results are largely consistent with X-ray microanalysis results from the pilocarpine-stimulated dog submandibular gland.

INTRODUCTION

There is currently much interest in detailing the secretion-related changes in intracellular concentrations of exocrine glands because of their fundamental roles in secretion processes. Numerous studies utilizing radioisotopes, flame photometry, and ion-specific and other electrodes have shown the importance of Ca, Na, K, Cl and HCO_3 ions to secretion. The electron microprobe constitutes a technique that is capable of yielding information about subcellular elemental concentrations that complements information from the preceding types of studies.

Hall (1971) formulated the theory of continuum normalization for the quantification of X-ray data from biological specimens, and Hall, Anderson & Appleton (1973) for thin sections of soft tissues. Gupta, Hall & Moreton (1977) provided a detailed exposition of the procedures and history as well as fully quantitative data from many tissues. Somlyo, Somlyo & Shuman (1979) confirmed the validity of Hall's method for ultra-thin cryosections in the freeze-dried state. Gupta & Hall (1983) used the technique to study cockroach salivary glands; Sasaki, Nakagaki, Mori & Imai (1983)

and Roomans, Wei, Ceder & Kollberg (1982) to study mammalian submandibular salivary glands; and Muller & Roomans (1984) to study the resting rat parotid gland. The present communication constitutes the first application of this method to the study of secretion-related, elemental concentration changes in the parotid gland. Pilocarpine was utilized as secretagogue in order to facilitate comparisons with previous microprobe secretion studies (Nakagaki, Sasaki, Shiguma & Imai, 1984; Sasaki *et al.* 1983) and because the drug was previously found to be suitable for electrolyte and fluid secretion studies in salivary glands (Schneyer & Hall, 1965). We report significant pilocarpine-related changes in electrolyte levels in secretory granules, cytoplasm and nuclei of parotid acinar cells. These changes have important physiological implications.

METHODS

Male Sprague–Dawley rats weighing about 300 g were anaesthetized with intraperitoneal (I.P.) sodium pentobarbitone at 60 mg/kg body weight. Subsequent doses of 20 mg/kg were utilized if needed to insure attainment of surgical anaesthesia. Salivation was induced with an I.P. injection of pilocarpine at 5 mg/kg. Copious salivary flow was usually observed within 3–8 min. Either parotid gland was then exposed, and small pieces of tissue no more than 2 mm in the longest dimension were dissected, placed on stainless-steel chucks, frozen by quick immersion in rapidly-stirred Freon 22 cooled with liquid nitrogen (−156 °C), and stored in liquid nitrogen until sectioned. Hence, samples were obtained between 5 and 10 min after pilocarpine injection. The time course of salivary flow under these conditions was established in a separate series of experiments with four animals using the present protocols except that the parotid duct was cannulated as previously described (Rice, Izutsu, Truelove, Anderson, Morton & Siegel, 1982). Salivary flow rates relative to the maximum rate in each animal were plotted as a function of time after injection, and averaged over all animals in two minute intervals. The results showed that parotid salivary flow reached a maximum at about 8 min after pilocarpine injection and then slowly decreased to a half-maximal flow rate at 30 min (Fig. 1). Thus, all samples were obtained during periods of maximal salivary flow. Samples from non-stimulated animals were obtained beginning at about 8 min after saline injection.

A Sorvall MT2-B cryo-ultramicrotome (E. I. Dupont de Nemours and Co., Claremont, CA, USA) was used to prepare thin sections. The cold bowl temperature was maintained at −100 °C. A knife clearance angle of 7 deg and lowest cutting speed were used. Dry cut sections were transferred to copper double grids coated with formvar and carbon films, and freeze-dried overnight at 10^{-5} torr in an Edward's high vacuum evaporator (Edward's, El Sugundo, CA, U.S.A.). Grids were stored in a desiccator until analysed.

Elemental analysis

The freeze-dried sections were examined in a JEOL-100C electron microscope (JEOL U.S.A. West, Burlingame, CA, U.S.A.). All X-ray spectra were collected in the scanning transmission mode at 20 °C. A square raster 1000 Å on a side, a beam current of 10^{-8} A and a counting time of 200 s for a total specimen dose of about 2×10^4 C/cm^2 were typically used. Specimens were tilted toward the detector at an angle of 45 deg from the incident beam, and the area analysed was about 2 cm from the detector. Analyses were made with the folding grids opened after freeze-drying and the specimen continuum count corrected for the continuum contribution of a single support film. Emitted X-rays from various cell structures were detected with a 30 mm^2 Si(Li) crystal, and X-ray energy spectra were generated with a Kevex 7000 pulse height analyser. The spectra were analysed with a dedicated PDP-11 microcomputer using a digital filtration and least-squares fitting program (Shuman, Somlyo & Somlyo, 1976). The analysis uses the ratio of peak counts to continuum (1·00–3·90 keV in our case) count to calculate elemental mass fractions as described by Hall *et al.* (1973). Binary salt standards were used to establish relative counting efficiencies, and S in an albumin sample was used as an absolute standard. The remaining absolute proportionality constants were then obtained using the relative proportionality constants. The resulting concentrations and associated experimental conditions were stored and analysed in a CDC Cyber 170–750 mainframe computer.

Mass loss

Mass loss was evaluated as previously described (Izutsu, Johnson, Schubert, Wang, Ramsey, Tamarin, Truelove, Ensign & Young, 1985). Briefly, a series of 25 s (live time) counts for each of the elements and the continuum were obtained. The initial count was typically obtained with a dose of about $2{\cdot}6 \times 10^{-4}$ C/cm^2, and the final accumulated dose was about $2{\cdot}7 \times 10^{3}$ C/cm^2. Care was

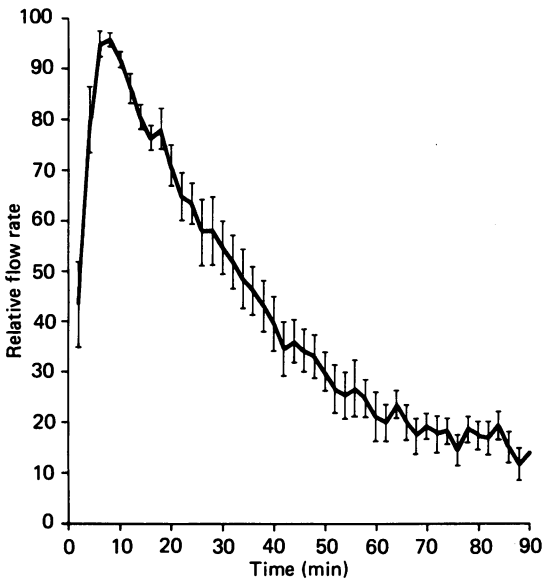

Fig. 1. Relative rat parotid gland flow rate as a function of time after intraperitoneal injection of pilocarpine. Values are means ±S.E. of mean from four rats.

taken to monitor for contamination, sample drift and shrinkage, and current drift. Mass loss was evaluated by regressing continuum (or specific elemental) counts against dose. Little or no mass loss was detected for doses between $2{\cdot}6 \times 10^{-4}$ and $4{\cdot}5 \times 10^{-3}$ C/cm^2. Between $4{\cdot}5 \times 10^{-3}$ and 1·0 C/cm^2, there was an ~ 40% mass (i.e. continuum count) loss. At higher doses, there was no further loss. S counts decreased in a similar fashion as the continuum counts, but the final amount of S loss was 75%. No significant elemental losses were detected for the other elements. Since all spectra were obtained with exposures greater than 1·0 C/cm^2, the calculated concentrations were adjusted accordingly.

Statistical analysis

Elemental concentrations from resting and salivating animals were compared using the unpaired *t* test between means of the means from each rat segregated by secretion status. *t*-test results were confirmed with the Mann–Whitney *U* test (Nie, Hull, Jenkins, Steinbrenner & Bent, 1975). One-tailed tests were utilized in those instances in which directional changes in concentration were predicted on the basis of observations from other laboratories (Zar, 1984). Tests for significant differences between organelles utilized the one-way analysis of variance and the Student–Neuman–Keuls method (Nie *et al.* 1975) for determining the least significant differences (again, using means of means). Correlations between the elemental concentrations and the various experimental parameters (e.g. film continuum counts, χ^2 of fit, Cu count, etc.) were tested using regression analysis. Statistical significance was assumed at the 0·05 level. All the above tests were done using the SPSS (Statistical Package for the Social Sciences) package (Nie *et al.* 1975). Calculations of the power (i.e. one minus the probability of making a type II error) of the analysis of variance tests were done by the method of Zar (1984).

RESULTS

Resting acinar cells

Acinar cells were readily identified in freeze-dried cryosections as were nuclei, secretory granules and cytoplasmic spaces (Pl. 1). X-ray spectra obtained from

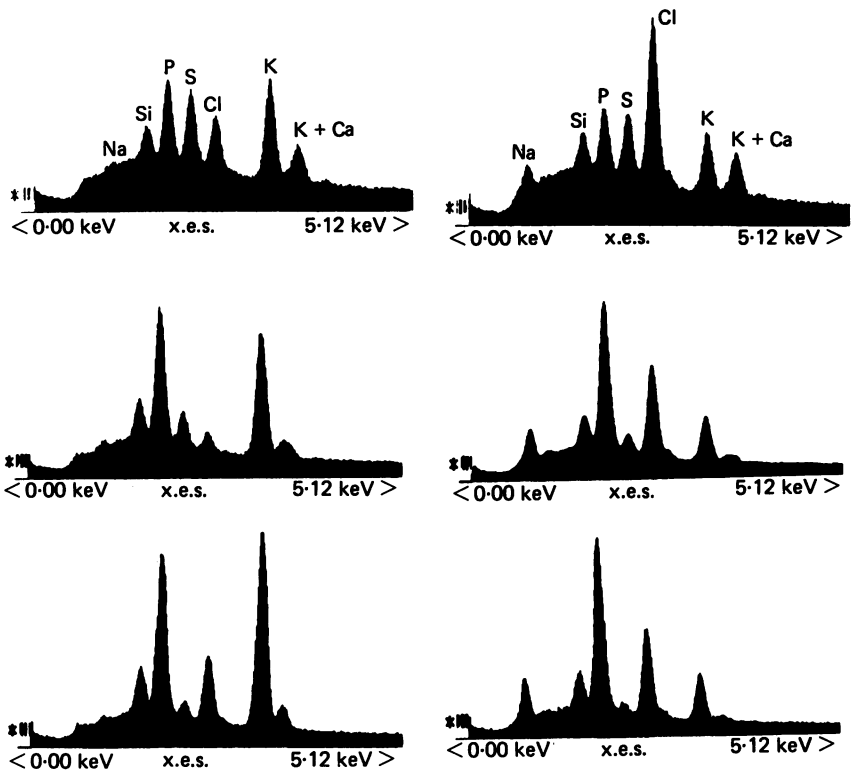

Fig. 2. Representative X-ray spectra obtained from secretory granules (top row), cytoplasm (middle row) and nuclei (bottom row) in parotid gland cryosections prepared from resting (left) and pilocarpine-stimulated (right) animals.

secretory granules were distingishable from those from cytoplasm and nuclei. Spectra from secretory granules typically consisted of nearly equally prominent P, S, Cl and K peaks, a slightly lesser Ca peak, a considerably smaller Mg peak and a small Na peak (Fig. 2). X-ray spectra from cytoplasm and nuclei typically consisted of large P and K peaks, a lesser Cl peak, and small peaks of Na and Mg (Fig. 2).

The calculated elemental concentrations reflected the above patterns (Table 1). Analyses of these concentrations showed several statistically significant differences between secretory granules and cytoplasm and nuclei of resting acinar cells. Cytoplasm and nuclei had significantly higher concentrations of Na, Mg, P, Cl, and K, and lower concentrations of S and Ca than secretory granules (Table 1). The negative concentration for nuclear Ca concentrations arises from fitting errors in the quantitation routine and does not have physiological implications other than that

TABLE 1. Elemental concentrations in organelles from resting and salivating rat parotid glands

Organelle state	Animals/ spectra	Concentrations (mmol/kg dry mass) Na	Mg	P	S	Cl	K	Ca
Secretory granule (s.)								
Resting	5/43	13±5*	31±5	152±17	536±12	97±6	151±23	69±11
Salivating	13/68	137±35	36±4	142±18	636±40	160±25	145±16	77±6
$P(t)$		0·004	N.s.	N.s.	0·04	0·02	N.s.	N.s.
Cytoplasm (c.)								
Resting	6/50	51±11	66±7	680±34	244±32	179±19	608±28	9±2
Salivating	15/170	326±40	83±5	701±32	272±16	182±11	436±34	14±2
$P(t)$		0·0001	N.s.	N.s.	N.s.	N.s.	0·007	0·03†
Nucleus (n.)								
Resting	6/44	49±13	57±10	749±34	148±24	210±19	840±37	−2±4
Salivating	14/74	361±66	70±5	829±56	196±16	196±13	551±65	2±1
$P(t)$		0·001	N.s.	N.s.	N.s.	N.s.	0·001	N.s.
Homogeneous groups‡								
Resting	—	s./c.,n.	s./c.,n.	s./c.,n.	s./c./n.	s./c.,n.	s./c./n.	s./c.,n.
Salivating	—	s./c.,n.	s./c.,n.	s./c./n.	s./c./n.	s.,c.,n.	s./c./n.	s./c./n.

$P(t)$ = significance of t tests between resting and salivating concentrations.
N.s. = Not significant.
* Values are means of means for each animal ±S.E. of mean.
† One-tailed test.
‡ Groups whose means differ by more than the least significant difference as calculated by Student–Neuman–Keuls, e.g. s./c.,n. signifies mean of secretory granule (s.) is significantly different from that in cytoplasm (c.) and nucleus (n.).

the value is quite low, i.e. less than about 6 mmol/kg dry mass (mean+2 S.E. of mean).

The only significant differences between cytoplasmic and nuclear concentrations were that S was lower and K was higher in nuclei (Table 1).

Pilocarpine-related concentration changes

Distinct changes in X-ray spectra were noted in glands exposed to pilocarpine. Na and Cl peaks increased in spectra from secretory granules (Fig. 2), and Na peaks increased and K peaks decreased in spectra from cytoplasm and nuclei (Fig. 2). Quantitative analyses showed secretory granule levels of Na increased tenfold, S by 18% and Cl by 66% (Table 1). Cytoplasmic and nuclear Na concentrations increased by over sixfold and sevenfold, respectively, while cytoplasmic and nuclear K concentrations decreased by about 30% and 35%, respectively. Cytoplasmic Ca increased by 56%.

Concentration differences in organelles during secretion

Analyses of elemental concentration differences between organelles showed two salivation-related changes. Whereas cytoplasmic and nuclear Ca and P concentrations were not significantly different from one another in resting acinar cells, they were significantly different in salivating cells (Table 1).

DISCUSSION

Concentration differences in organelles of resting glands

The present results indicate that Na, Mg, P, K and Cl dry mass concentrations are significantly lower and that Ca and S concentrations are significantly higher in secretory granules than in cytoplasm or nuclei (Table 1). The high Ca concentration in secretory granules is qualitatively consistent with previous results of Wallach & Schramm (1971). Wallach (1982) estimated that the total Ca concentration in secretory granules exceeds 10 mM by assuming that the content of isoprenaline-stimulated saliva is close to that inside the granules. This approximation is in good agreement with the value of 23 ± 2 mM that can be calculated from the present dry mass Ca concentration using the dry mass fraction for submandibular gland secretory granules of 32·7% found by Sasaki *et al.* (1983). This value is close to the value of 37% found by Nakagaki *et al.* (1984) for the secretory granule of the pancreatic serous acinar cell which shares many characteristics of the secretory granule of the rat parotid acinar cell. However, data are still lacking on the dry mass fraction of the rat parotid gland secretory granule *per se*. The present S finding in secretory granules is consistent with the known incorporation of S into secretory macromolecules in the golgi region and their subsequent transport into secretory granules (Reggio & Palade, 1975; Berg & Austin, 1976). The high P levels in cytoplasm and nuclei relative to secretory granules can probably be attributed to high RNA (cytoplasmic and nuclear) and DNA (nuclear) levels, the former probably being associated with secretory protein synthesis (Palade, 1975; Wallach, 1982).

Two statistically significant differences were found between resting cytoplasmic and nuclear elemental concentrations. S concentration was significantly lower and

K concentration was significantly higher in nuclei *versus* cytoplasm. The higher S concentration in cytoplasm may be attributed to sulphation of secretory products. The reason for the higher nuclear K levels is unknown. It might be accounted for by differences in hydration between cytoplasm and nuclei. Even though a statistically significant difference in the average nuclear and cytoplasmic dry mass contents as measured by continuum counts could not be demonstrated, the results were not definitive due to the low statistical power of the test. These intracellular differences are similar to those reported by Somlyo *et al.* (1979) in vascular smooth muscle except they also found Cl to be significantly lower in nuclei. In addition, Somlyo *et al.* hypothesized that some nuclear K may be associated with non-diffusible negative charges in the nuclear matrix. Significant nuclear to cytoplasmic K dry mass concentration gradients have also been reported in other tissues from several species (e.g. Jones, Johnson, Gupta & Hall, 1979). However, studies utilizing ion-specific electrodes and nuclear magnetic resonance showed no such gradients for ionic K (Civan, 1980). Hence, the present dry mass concentration difference may not reflect a difference in K molar activities in the two intracellular compartments.

The present cytoplasmic results correspond to cytosolic concentrations of 13 ± 8, 152 ± 18 and 45 ± 11 mM for Na, K and Cl, respectively, based on a dry mass fraction of 20% (Sasaki *et al.* 1983). These values are in good agreement with the results of Schneyer & Schneyer (1960) based on flame photometry titration and tissue compartment measurements (Na = 22, K = 142 and Cl = 48 mM).

Salivation-related concentration changes

The principal pilocarpine-related finding is that cellular Na concentration increased and cellular K concentration decreased with pilocarpine treatment (Table 1). A secretion-related increase in Na content is consistent with previous reports (Burgen, 1967; Schneyer, Young & Schneyer, 1972; Putney & Parod, 1978). However, the present results considerably extend our knowledge about this uptake since information is provided about Na and other elemental concentration changes in several intracellular compartments. In particular, cytoplasmic Na concentration increased while that of K decreased, and similar changes were found in the nucleus except the stoicheiometry of the Na and K changes appeared to be one-to-one. Na concentration was also elevated in secretory granules, but this was accompanied by an increase in Cl concentration rather than a decrease in K concentration as observed in the other compartments.

Quissell (1980) and Poulsen & Bledsoe (1978) previously noted that the secretion-related changes in K concentration depend on the experimental preparation. Isolated cells rapidly lose (within 1 min) and regain (within 5 min) K, while cells in intact glands or in gland slices maintain the initial K decrease at long times after onset of stimulation. Hence, the rate of release and re-uptake of K ions might depend on the clearance and delivery of K ions to the cells (Poulsen & Bledsoe, 1978; Poulsen & Oakley, 1979). Since cytoplasmic and nuclear K levels were still significantly decreased at the long times used in this study, the present results are similar to those obtained in gland slices and intact glands as expected. In addition, the magnitude of the changes are similar: 28% in the present cytoplasm, 20% in submandibular gland slices after 10 min by flame photometry (Martinez & Quissell, 1976), and 30%

by K ion specific electrode (Poulsen & Oakley, 1979). The present finding of nuclear Na and K exchange following pilocarpine stimulation may be of physiologic importance since changes in electrolyte concentrations can affect nuclear processes. For example, depletion of nuclear K arrests cell division, and changes in electrolyte levels lead to gross changes in chromatin condensation and in deoxyribonucleo-protein fibril dimensions (Jones *et al.* 1979). It is tempting to speculate that the secretion-related nuclear electrolyte changes might regulate nuclear processes involved in the synthesis of secretory proteins.

Finally, it should be stressed that since the above results are dry weight measurements, no inferences are made concerning cell water changes following stimulation. Equally important, however, is the fact that all conclusions reached regarding elemental changes following stimulation are independent of any such cell water changes.

Comparisons with previous X-ray microanalysis results

The present elemental concentrations are in qualitative agreement with values obtained by other researchers using the analytical electron microscope. Comparison with the only previous study of the resting rat parotid gland (Muller & Roomans, 1984) shows the same relative relationships between elements as reported here although the absolute concentrations differ significantly for elements present in low concentrations (Tables 2–4). The cytoplasmic values of Muller & Roomans (1984) are not directly comparable with present values since the former were obtained in 16 μm sections while ours were obtained with significantly thinner sections (Table 2). (Although we have not measured this thickness *per se*, our sections are of golden colour which would correspond to a thickness of about 1200 Å for conventionally prepared material.) The relative nuclear concentrations agree with present values in the sense that both studies found Ca to be of lowest concentration, Na, Mg, S and Cl to be of intermediate concentration and P and K to be of highest concentration (Table 3). The difference in absolute P concentrations may be attributable to a non-homogeneous nuclear distribution of P (K. T. Izutsu & D. E. Johnson, unpublished results). The reason for the difference in K concentrations is unknown. The relatively good agreement in P, S and Cl values between the two studies makes calibration error unlikely. The fact that Na concentration is elevated while K concentration is reduced suggests the cells in the previous study may have been slightly depolarized., Comparison of elemental concentrations in secretory granules in the two studies shows major differences in P, S, K and Ca values (Table 4). Since the values for these elements from the latter study are closer to cytoplasmic values than are the values from the present study, it is likely that these differences arise from varying amounts of cytoplasmic contribution to secretory granule spectra. However, the possibility remains that secretory granules of varying maturity were sampled in the two studies. Only large, dense, apically located granules were sampled here.

Comparison of the present findings with those from other previous X-ray microanalysis studies are complicated by gland and species differences. None the less, the submandibular gland acinar cytoplasmic results of Sasaki *et al.* (1983) are in good agreement with present findings in that both studies found Na to increase to about

TABLE 2. Resting and cholinergically stimulated cytoplasmic elemental concentrations in secretory cells of different glands

Tissue state	Reference	Concentrations (mmol/kg dry mass) Na	Mg	P	S	Cl	K	Ca
Rat parotid acini	Muller & Roomans (1984)							
Rest		114±16*	36±2	633±25	197±8	188±10	508±26	20±1
Dog submandibular mucous acini	Sasaki *et al.* (1983)							
Rest		125±60†	—	—	—	105±55	575±185	—
Stimulated		285±95	—	—	—	270±105	380±130	45±25
Rat submandibular acini (slices)	von Euler, Ceder, Kollberg & Roomans (1985)							
Rest		218±24*	92±3	430±12	124±12	191±20	373±26	46±5
Stimulated		235±21	95±12	447±23	131±9	166±10	382±25	48±7
Dog pancreatic acini	Nakagaki *et al.* (1984)							
Rest		22±10†	—	750±163	66±13	64±21	600±68	—
Stimulated		68±29	—	732±164	77±14	114±33	541±68	17±6
Cockroach P cell	Gupta & Hall (1983)							
Rest		80±10*	24±4	570±50	240±20	235±15	440±15	5±3
Stimulated		76±4	19±4	632±8	244±24	192±8	532±4	4±2

* Means ±S.E. of mean.
† Means ±S.D.

TABLE 3. Resting and cholinergically stimulated nuclear elemental concentrations in secretory cells of different glands

Tissue state	Reference	Concentrations (mmol/kg dry mass) Na	Mg	P	S	Cl	K	Ca
Rat parotid	Muller & Roomans (1984)							
Rest		128±13*	30±4	983±49	157±8	242±21	456±23	16±5
Rat submandibular	Muller *et al.* (1985)							
Rest		143±51*	78±8	802±117	215±48	182±27	599±62	25±9
Cockroach P cells	Gupta & Hall (1983)							
Rest		50±10*	11±4	860±30	240±15	145±20	790±25	6±3
Stimulated		70±4	22±4	656±13	114±7	189±4	633±11	11±4

* Means ±S.E. of mean.

TABLE 4. Resting and cholinergically stimulated elemental concentrations in secretory granules in secretory cells from different glands

Tissue state	Reference	Concentrations (mmol/kg dry mass) Na	Mg	P	S	Cl	K	Ca
Rat parotid	Muller & Roomans (1984)							
Rest		67±6*	30±3	636±33	214±8	202±9	357±14	42±3
Dog submandibular	Sasaki *et al.* (1983)							
Rest		108±51†	—	—	—	201±72	165±42	147±48
Stimulated		222±93	—	—	—	261±87	213±72	96±45
Dog pancreas	Nakagaki *et al.* (1984)							
Rest		16±14†	—	97±22	465±18	84±54	162±43	19±14
Stimulated		24±19	—	89±16	446±76	103±46	146±32	16±11
Human labial gland	Izutsu *et al.* (1985)							
Rest		41±3*	37±14	95±10	248±40	91±17	296±38	86±8
Rat submandibular	Muller *et al.* (1985)							
Rest		75±10*	86±6	577±30	154±9	158±13	522±22	53±7

* Means ±S.E. of mean
† Means ±S.D.

300 mmol/kg dry mass and K to decrease to about 400 mmol/kg dry mass following pilocarpine stimulation (Table 2). Similar Na and K changes but of smaller magnitude were observed in dog pancreatic acini (Table 2). This difference could arise because pancreatic flow was not maximal at the time of study, i.e. at 1 min after pilocarpine injection, because the pilocarpine dose (1 mg/animal) was not maximal, or because the mechanism of fluid secretion may be different in salivary glands and the pancreas. Specifically, Young, Chapman, Cook, Healey, Kuchel, Lingard, Nicol, Novak & Seow (1982) showed fluid production is sensitive to perfusion pressure in the pancreas but not in the submandibular gland. Hence, hydrostatic pressure and not osmotic coupling may underlie pancreatic but not salivary fluid formation.

Cytoplasmic Ca concentration was also found to increase in all three tissues following pilocarpine, but an increased cytoplasmic Cl concentration was only found in dog submandibular and pancreatic acini (Table 2). This stimulation-related increase in cytoplasmic Ca concentration is consistent with the hypothesis that Ca is an intracellular second messenger controlling electrolyte and water fluxes (O'Doherty, Stark, Crane & Brugge, 1983). However, the time resolution of the present and previous microprobe studies was not adequate to determine whether the Ca changes preceded the K and Na changes. In addition, O'Doherty *et al.* (1983) measured a secretion-related change in cytoplasmic Ca ion concentration of about 600 nM with a Ca-sensitive micro-electrode in cholinergically stimulated mouse parotid acini *in vitro*. The present secretion-related change in cytoplasmic total Ca dry mass concentration corresponds to a total Ca concentration change of 1·25 mM, which is over three orders of magnitude larger than the above ionic Ca change. These results indicate that acinar cytoplasmic Ca buffering capacity is similar in magnitude to that reported for squid axoplasm (Baker & DiPolo, 1984).

Secretory granules from parotid, submandibular and pancreatic acini all were found to have increased Na and Cl concentrations following pilocarpine stimulation, but submandibular secretory granules also had increased K and decreased Ca concentrations (Table 4). Since serous and mucous secretory granules are known to contain very different secretory products, it is not surprising that they would also have different elemental concentrations. Comparison of the parotid results with those from mucous secretory granules of the human labial gland (Table 4) suggests that a major difference may be in S content (Izutsu *et al.* 1985).

Examination of the cytoplasmic microprobe results from rat submandibular gland slices incubated *in vitro* shows cells in these slices had marginally increased concentrations of Na and K following stimulation with carbachol (2×10^{-5} M) (Table 2). This finding probably results from inadequate *in vitro* incubation conditions since Martinez & Quissell (1976) showed a large cholinergically induced release of intracellular K from this preparation using flame photometry.

Comparison of present results with those from P cells of the cockroach salivary gland is complicated by significant gland and species differences (Tables 2 and 3). However, qualitatively similar relative elemental concentrations are evident.

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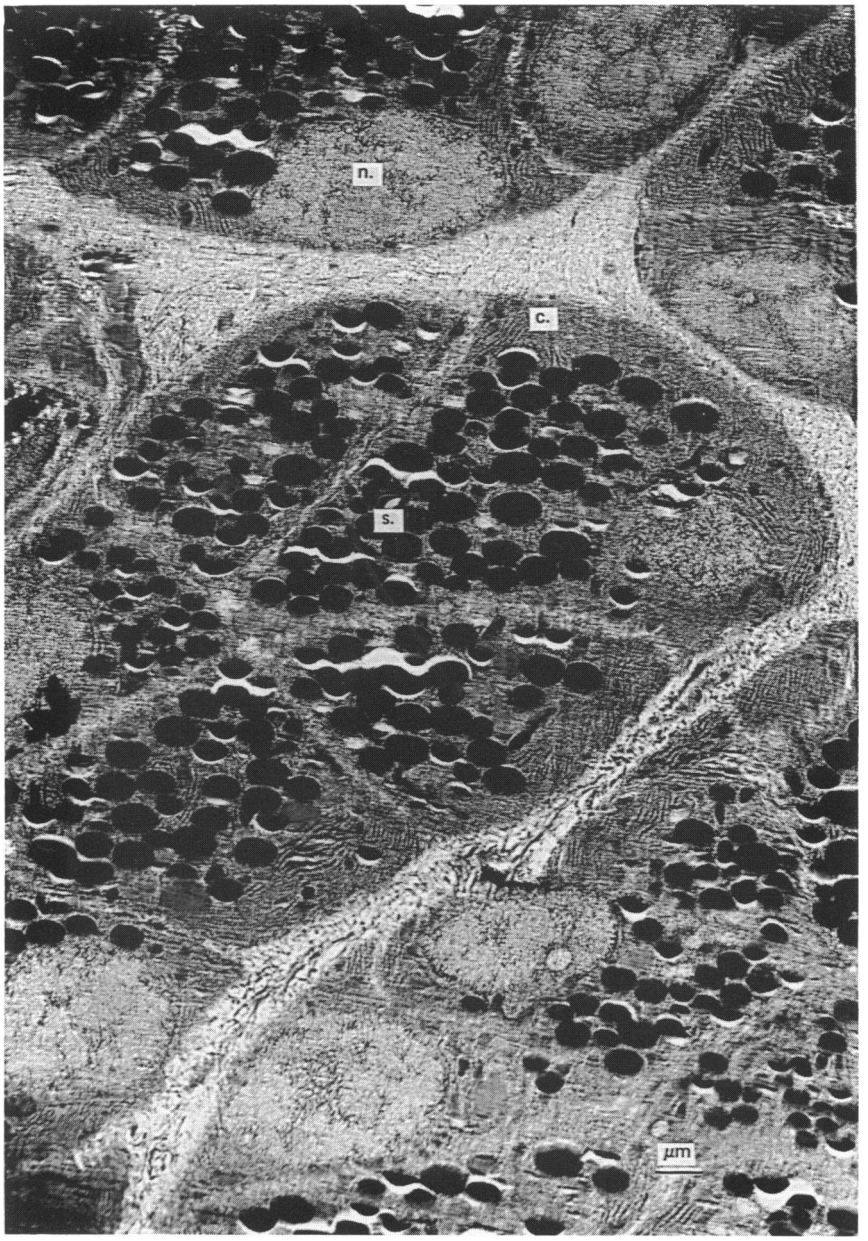
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EXPLANATION OF PLATE

Freeze-dried cryosection of rat parotid gland showing acinar cell secretory granules (s.), cytoplasm (c.) and nuclei (n.).