Effects of LiCl and LiCl plus methacholine on the intracellular composition of goose salt-gland slices

		Tissue			
		A Incubated control	B Incubated + 10 m*M* LiCl	C Incubated + 10 m*M* LiCl + 0.33 m*M* methacholine	*P* value for paired *t*-test between B and C
		(11)	(7)	(7)	
Water content (ml/100 g tissue)		76.7 ± 0.9	77.6 ± 0.9	78.0 ± 1.2	NS
^{14}C-sucrose space (ml/100 ml tissue water)		57.3 ± 2.1	57.8 ± 1.9	54.8 ± 2.8	NS
Calculated intracellular concentration	Na	58.3 ± 1.9	78.8 ± 7.0 $p < 0.01$	62.8 ± 6.5	< 0.05 (MD = 16)
(mmoles/l intracellular water)	K	112.0 ± 9.4	127.0 ± 2.0	124.9 ± 8.2	NS
	Cl	63.4 ± 4.8	84.9 ± 10.2 $P < 0.05$	68.8 ± 10.9	< 0.05 (MD = 16)
	Li	—	11.7 ± 1.5	12.0 ± 2.1	NS

Lithium (10 m*M*) and methacholine (0.33 m*M*) were added for the final 10 or 20 min of incubation as described in the text. Data from both times of incubation have been bulked since there was no apparent difference between them. Mean ± S.E.
Number of determinations in parentheses. The *P* values below the figures in the columns are for a *t*-test between the data in B or C compared with the control data in A. NS = not significant (only shown for the paired *t*-test). MD = mean difference.

were identical (16 mmoles/l). This finding implies that, under these conditions, Na^+ and Cl^- movements were affected equally while intracellular [K] remained unchanged. There was no significant difference in intracellular [Li] between the two groups incubated with LiCl or LiCl plus methacholine, and no significant correlation was apparent between intracellular [Li] and [Na] in the slices incubated with 10 m*M*-LiCl.

The increases in intracellular [Na] and [Cl] with LiCl can be interpreted as providing further evidence for the hypothesis that, during secretory activity, Na^+ and Cl^- are transported into the cell across the basal and lateral membranes in exchange for H^+ and HCO_3^-. The 20% increase in cell respiration with Li^+[3] (which we confirm) would lead to more CO_2 (and therefore H^+ and HCO_3^-) being available for these exchanges to occur; the mechanism by which Li^+ exerts its effect on respiration is not known. The equimolar decrease in intracellular [Na] and [Cl] which occurred when methacholine was also present implies that this cholinomimetic activates the sodium pump on the luminal membrane which then extrudes the additional Na^+ and Cl^- entering the cell under the influence of Li^+. In other words methacholine restored the balance between influx and efflux across the two poles of the secretory cell. It would also seem unlikely that a rise in intracellular [Na^+] is the means by which the luminal sodium pump is activated because, when Li^+ was present, intracellular [Na^+] was increased and yet methacholine still appeared to stimulate the luminal pump. A similar suggestion, that a raised intracellular [Na^+] is not responsible for the activation of the luminal pump in the cat submandibular gland, has been made recently[6]. If this interpretation is correct, the question arises, how does acetylcholine, released from the secretory nerve terminals at the basal side of the cell, stimulate the pump on the opposite (i.e. luminal) side of the cell?

While these findings on the effect of Li^+ provide useful information on the secretory mechanism in the salt gland, the implications may be of wider interest. It is possible that Na^+ and Cl^- movements could be affected by Li^+, acting to alter cell respiration, in epithelial tissues with Na^+/H^+ and Cl^-/HCO_3^- exchange mechanisms on one border of the cells. Since such tissues apparently exist in mammals as well as in other vertebrates[7] and, with the current interest in lithium as an agent for use in the treatment of psychological disorders and in any side effects it may have, such a possible mechanism of action should not be overlooked.

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Effect of a Chelating Ion Exchange Resin (Chelex 100) on Impedance and Evoked Potentials

Both raising and lowering calcium levels in the environment of nervous tissue has long been known to alter its excitability[1-5]. Changes in impedance of cerebral cortex after topical application of calcium and magnesium solutions were previously reported[6-8]. Topical application of ion chelating gels produced inversion of the transcortical DC gradient, EEG flattening, and seizure activity[9].

In the present note we report the effects of a chelating ion exchange resin Chelex 100 (Bio Rad Laboratories) on flash evoked potentials and impedance. Chelex 100 is a styrene divinylbenzene copolymer containing imminodiacetate functional groups. It is structurally classed with the weak acid cation exchangers by virtue of its carboxylic acid groups. Its high affinity for calcium and magnesium makes it suitable for studying effects of removal of these ions from nervous tissue.

The study was performed in 32 alert cats with chronically implanted cannulae and bipolar concentric electro-

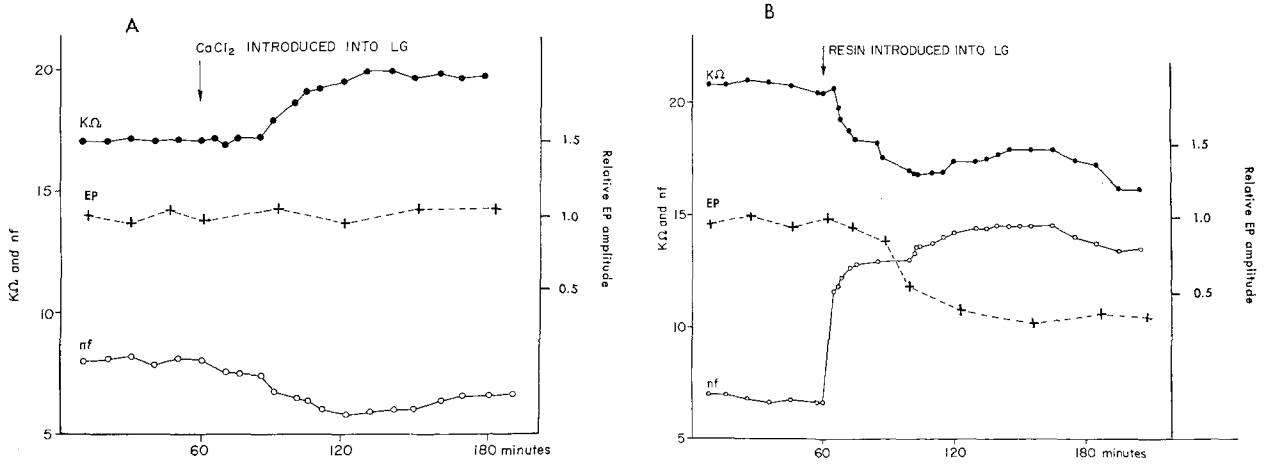

Fig. 1. A) Effect of calcium chloride on lateral geniculate body impedance and flash evoked potentials (EP). Arrow indicates introduction of 5 µl $CaCl_2$ 200 m*M* into the lateral geniculate body of the cat. B) Effect of ion exchange resin (Chelex 100) on lateral geniculate body impedance and flash evoked potentials (EP). Arrow indicates introduction of 1–2 mm^3 resin into the lateral geniculate body of the cat. ●, resistance; ○, capacitance; +, relative evoked potential amplitude.

des in the lateral geniculate body (LG). The electrodes and 22-gauge stainless steel cannulae were attached together so that their tips were at the same height, but 1–2 mm apart (as described previously[10]). EEG evoked potentials to flash stimuli and electrical impedance at 1.0 kHz with low current levels (10^{-13}Amp μM^{-2} of electrode surface) were recorded from the electrodes.

Figure 1A shows that introduction of 5 µl 200 m*M* $CaCl_2$ into LG resulted within 20 min in an increase in reactive impedance (decrease in capacitance). Those changes persisted for 3 h. Similar results were obtained in 5 other experiments. No changes in evoked potentials were observed.

Figure 1B shows the time course of the effects of the chelating ion exchange resin (Chelex 100) when 1–2 mm^3 of the agent was introduced into the LG. Within 10 min a drop in reactive impedance (increase in capacitance) and decrease in flash evoked potentials was observed. The changes persisted for 24 h. Similar results were obtained in 8 other experiments: no effect was recorded in 2 experiments.

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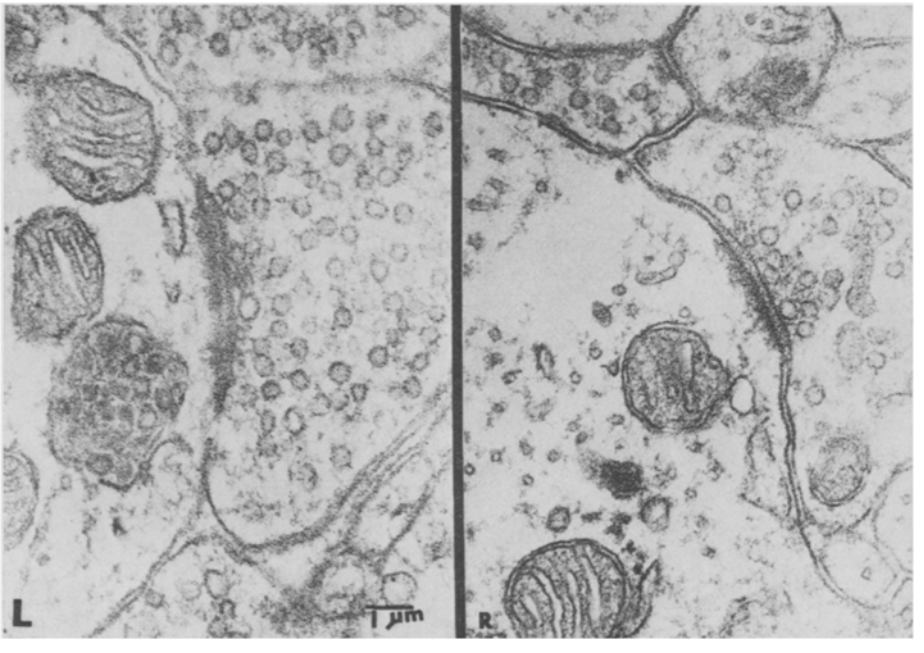

Fig. 3. Electron micrographs from the lateral geniculate body of the cat following treatment with chelating resin (L) or with resin with reduced chelating activity (R). Fixation by perfusion with phosphate-buffered glutaraldehyde-formaldehyde after flushing with physiological saline; post-fixation with buffered 1% OsO_4; dehydration with ethanol; embedment in EPON; sections enhanced with uranyl-acetate and lead citrate. ×30,000.

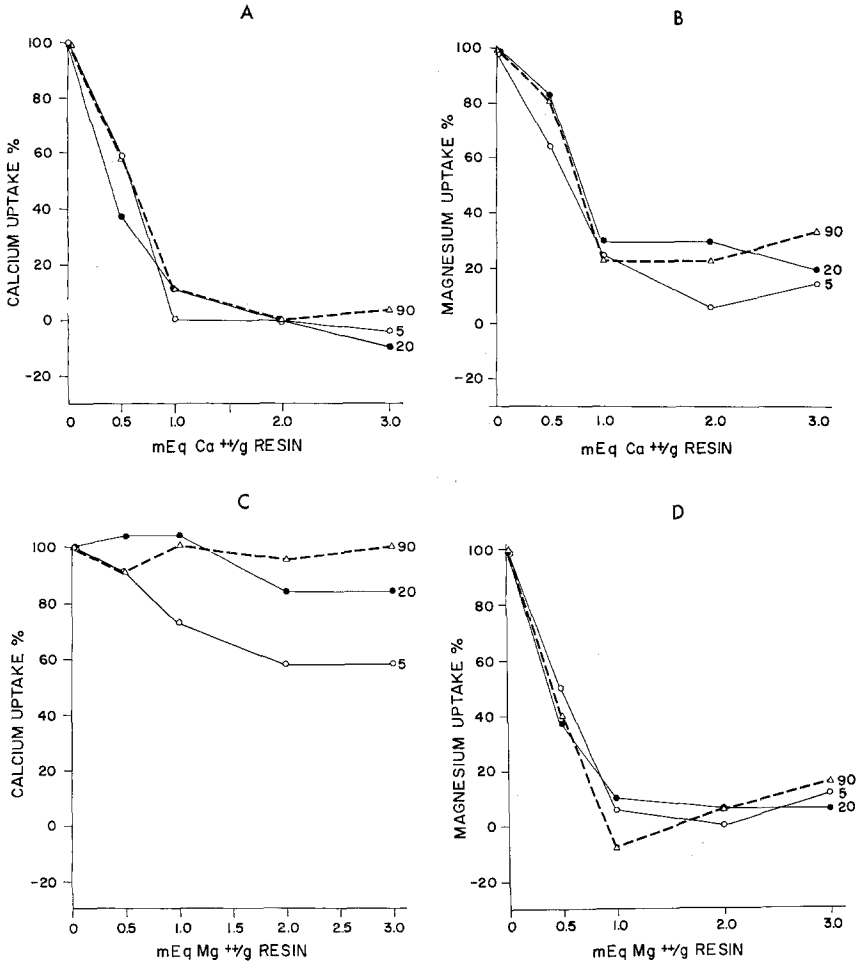

Fig. 2. Uptake of calcium (A) and magnesium (B) by pretreated chelating resin for 24 h with 0.5, 1.0, 2.0 and 3.0 mEq Ca^{++}/g resin (upper part). Uptake of calcium (C) and magnesium (D) by pretreated chelating resin for 24 h with 0.5, 1.0, 2.0 and 3.0 mEq Mg^{++}/g resin (lower part). To evaluate the uptake, each set of pretreated resin was subjected to the addition of 3 mEq of Ca^{++} or Mg^{++} and measurements using an atomic absorption spectrometer were made after 5 (○—○), 20 (●—●) and 90 (△—△) min.

No electrophysiological changes were observed if the same volume of artificial cerebrospinal fluid (5 experiments) was introduced into the lateral geniculate, or if prior to the application, the chelating capacity of the resin was reduced by prior equilibration (24 h) with 1–2 mEq per g resin for $ZnCl_2$ (2 experiments), $CaCl_2$ (6 experiments) or $MgCl_2$ (6 experiments). Prior equilibration with less than 1 mEq of $CaCl_2$ or $MgCl_2$ did not modify the electrophysiological effects of the resin.

Uptake of both Ca (Figure 2A) and Mg (Figure 2B) by the chelating resin drops dramatically to 5% when pretreated with 0.5, 1.0, 1.5, 2.5, 3.0 mEq Ca^{++}/g resin. Figure 2 shows also the uptake of calcium (2C) and magnesium (2D) by the chelating resin when pretreated with 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mEq Mg^{++}/g resin. The uptake of Mg drastically drops at 1 mEq, whereas the uptake of Ca decreases only slightly to 70% at (1 mEq) and 60% (2.0, 2.5, 3.0 mEq) indicating the higher affinity of the resin for Ca than for Mg.

The fact that not only the resin equilibrated with Ca, but also the resin equilibrated with Mg had no electrophysiological effect when introduced into the LG, might be explained by 1) its lower (60%) capacity of binding Ca^{++} and 2) the effect of the Mg^{++} dislocated by Ca^{++} from the pretreated resin.

To answer the question of the relation between those changes in evoked potentials and in tissue impedance and ultrastructural components, electron microscopy was performed on tissue taken from the lateral geniculate body in the zones adjoining sites of implanted electrodes and cannulae.

Figure 3 shows that following the application of the resin (3L) the intercellular spaces were not affected to any noticeable degree, but the synaptic cleft was altered – its membranes appeared fuzzier and less well defined than in the control (3R).

These findings offer support for the view that the electrophysiological effects attributable to depletion of calcium ion concentration in the extracellular space may relate to changes in the neuronal membrane structure, rather than to modifications in extracellular space.

The results contribute further evidence to the physiological role of divalent ions Ca and Mg in the neuronal membrane environment[11].

Résumé. L'introduction d'une résine chélatrice pour Ca^{++} et Mg^{++} (Chelex 100) dans le corps genouillé du chat produit une diminution des potentiels évoqués photiques et de l'impédance cérébrale. L'électronmicroscopie révèle une altération de la structure du champ synaptique sans modification de l'espace intercellulaire.

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