## THE RELATION BETWEEN INITIAL EQUATORIAL CELL DIAMETER AND CLEAVAGE CONSTRICTION RATE

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The reduction in equatorial diameter which comprises cytokinesis reflects the shortening of a circumferential band of contractile material, known as the contractile ring, which is contiguous to the cell surface. Since the cross-sectional area of the contractile ring remains constant, reduction in volume of ring material must occur *pari passu*. It is tempting to link the disappearance of ring material with force production and such linkage could imply that the force produced is a function of the rate at which the contractile ring diminishes. Previously published measurements revealed that the forces exerted by the furrows at first and second cleavages of sand dollar eggs are the same. However, the rates at which constriction takes place at first and second division are unequal, the first being significantly faster than the second in 2 species of echinoderms. In order to determine whether the rate differences were due to differences in division mechanisms or to simple geometrical differences, uncleaved sand dollar (*Echinarachnisus parma*) eggs were altered so that their diameters approximated that of one of the first 2 blastomeres before second cleavage, and the constriction rates were compared. The alteration was accomplished before fertilization, either by fragmenting eggs, or by extruding them into cylindrical form by expulsion through a pipette nozzle into a sperm suspension. In the latter eggs, cylindrical form was molded and maintained by the fertilization membrane. The results were as follows:

| | Initial Diameter | Rate of Constriction (μm/min) |
|---|---|---|
| First cleavage (normal) | 151.3 μm, s.d. 7.03 | 9.9, s.d. 2.18 |
| Second cleavage (normal) | 107.5 μm, s.d. 5.20 | 6.7, s.d. 1.209 |
| Fragments (spherical) | 103.6 μm, s.d. 12.4 | 6.44, s.d. 1.27 |
| Cylindrical whole eggs | 115.5 μm | 6.44 |

Results indicate that the first cleavage is inherently like that of the second, and the actual rate is related to initial equatorial diameter and thus to some geometrically related factor. It is simplest to propose that the rate is affected by the resistance of surface forces, which would be greater in smaller cells. This investigation was supported by National Science Foundation Grant PCM 74-18380A02 to R. Rappaport.

## ION MOVEMENTS IN THE DOGFISH RECTAL GLAND - EVIDENCE FOR THE INDEPENDENT UPTAKE OF SODIUM AND CHLORIDE

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It has been shown that at least part of the mechanism of cyclic AMP (cAMP) stimulation of secretion in the dogfish rectal gland involves an increase in the number of ouabain-binding sites in the tissue (Shuttleworth and Thompson, J. exp. Zool. 206:297-302, 1978 and this bulletin). The existence of an additional part of the mechanism involving modifications of the uptake of ions at the baso-lateral membranes is suggested by two facts: (1) In slices of *Scyliorhinus* rectal gland, cAMP increases ouabain-sensitive oxygen consumption to an extent ten times greater than its effect on the number of ouabain-binding sites (Shuttleworth and Thompson, J. comp. Physiol. - in press). This indicates that some

additional process is involved in the stimulation of overall pump activity other than an increase in pump sites *per se*. (2) It would seem logical that if cAMP was stimulating the active ion extrusion from the cells, then this might rapidly deplete the pool of transportable ions unless the rate of uptake of ions into the cells is also increased.

To investigate this, tissue slices 200-250 μm thick were obtained from freshly excised *Squalus* rectal glands using a mechanical tissue chopper and incubated in a shaking water bath at 15°C. Batches of slices from individual fish were incubated in 3 ml of dogfish saline, with or without the addition of 0.05 mMol $l^{-1}$ dibutyryl cAMP and 0.25 mMol $l^{-1}$ theophylline, for 45 mins. Ouabain was then added to each flask to give a final concentration of $10^{-4}$ M and incub ation continued for a further 30 mins. Samples of the slices were taken prior to incubation, after the first 45 mins and at the end of the experiment. These samples were blotted and weighed, dried overnight at 95°C, reweighed and digested in 0.1 N $HNO_3$ for 48 hours. The digest was then analyzed for $Na^+$ and $K^+$ (flame photometer) and $Cl^-$ (Corning-Eel chloridemeter). A parallel series of experiments was run in which slices were incubated under identical conditions to those described above except that $^{14}C$ polyethylene glycol (PEG) (0.1 μCi $ml^{-1}$) was added to the saline as a marker of the extracellular fluid compartment. Samples of the slices in these experiments were taken after the initial 45 mins period and at the end of the experiment, blotted and weighed, dried overnight at 95°C, reweighed and then solubilized (Soluene, Packard) and the activity corresponding to tissue PEG assayed by scintillation spectrometry. The data were expressed as μmol per g tissue dry weight (tissue ions) or ml per g dry weight (total tissue water and intracellular volume).

The results are illustrated in Figure 1. It can be seen from the values obtained after 45 mins that the presence of cAMP and theophylline in the incubation medium has no significant effect on any of the parameters measured. As discussed above, measurements of ouabain-sensitive oxygen consumption and ouabain binding have indicated that cAMP produces an increase in sodium pump activity. It follows that

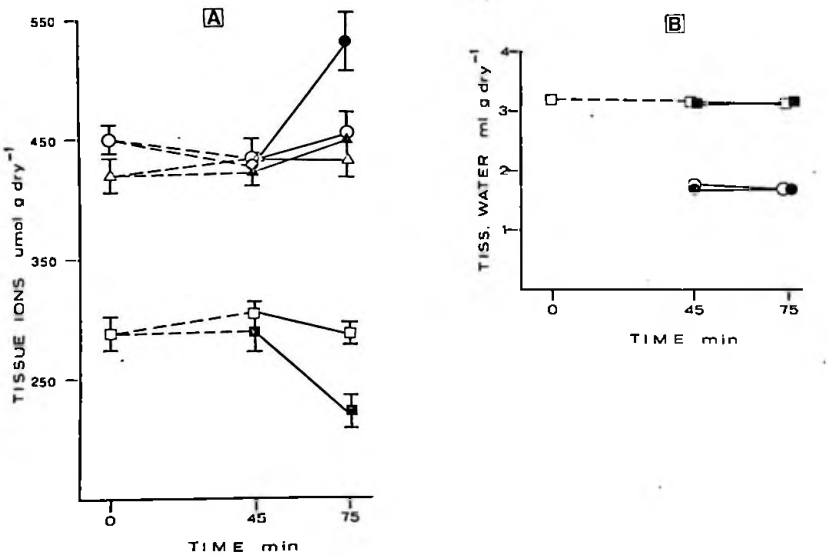


Figure 1. The effect of $10^{-4}$ M ouabain on tissue ions and water in untreated and cAMP/theophylline-treated slices of rectal gland. A: tissue ions - O ●, sodium; Δ ▲, chloride; □ ■, potassium. B: tissue water - □ ■, total water; O ●, intracellular water. Solid symbols represent cAMP/theophylline-treated tissue in each case.

as the presence of cAMP for a 45 min incubation period produces no significant change in tissue sodium levels (and total tissue and intracellular water spaces are the same in treated and control tissue),

this, in itself implies an increased rate of entry of sodium into the cells in tissue incubated in cAMP. This is confirmed by the continued incubation in the presence of $10^{-4}$ M ouabain. As would be expected from an inhibition of the sodium-potassium exchange pump, the addition of $10^{-4}$ M ouabain produces a rise in tissue sodium and fall in tissue potassium. However, the rate of these changes is far higher in the tissue treated with cAMP and theophylline. For example, tissue sodium increases some four times faster in the treated tissue (432 µmol g $dry^{-1}$ to 533 µmol g $dry^{-1}$ vs. 434 µmol g $dry^{-1}$ to 458 µmol g $dry^{-1}$). As there are no significant changes in total tissue water or intracellular volume during this period, the changes observed can only be explained by an increase in intracellular sodium (and a decrease in intracellular potassium). It is obvious, therefore, that the entry of sodium into the cells is greatly stimulated by cAMP and theophylline.

A widely accepted model for ion transport across the dogfish rectal gland has been proposed by Silva *et al.* (Am. J. Physiol. 233:298-306, 1977), a critical feature of which is a coupled sodium-chloride co-transport system located at the baso-lateral surface. This model proposes that the entry of chloride ions into the cell against their electrochemical potential is dependent on, and tightly coupled to, the entry of sodium ions down their electrochemical gradient. It is postulated that such coupling occurs via a common carrier with sites for both sodium and chloride ions. However, Figure 1 shows that the large increase in tissue sodium seen in cAMP-treated tissue following the addition of ouabain occurs without any corresponding increase in tissue chloride. This implies that, in the presence of cAMP, either (i) an increased entry of chloride into the cells (coupled to the increased sodium entry) is obscured by a simultaneous increase in the exit of chloride, or (ii) the cAMP-stimulated entry of sodium ions is independent of any chloride uptake. From experiments on perfused glands and isolated membrane vesicles (Silva *et al.*, Am. J. Physiol. 233:298-306, 1977; Eveloff *et al.*, Pflugers Arch. 378:87-92, 1978), it has been suggested that the proposed coupled sodium-chloride co-transport system is inhibited by furosemide and therefore this drug was used to test these two alternatives.

The experiments were carried out exactly as described above except that furosemide, to give a final concentration of 5 x $10^{-4}$ M, was added after 45 mins instead of ouabain. The results are presented in Figure 2. It can be predicted that, if cAMP is stimulating the coupled entry of sodium and

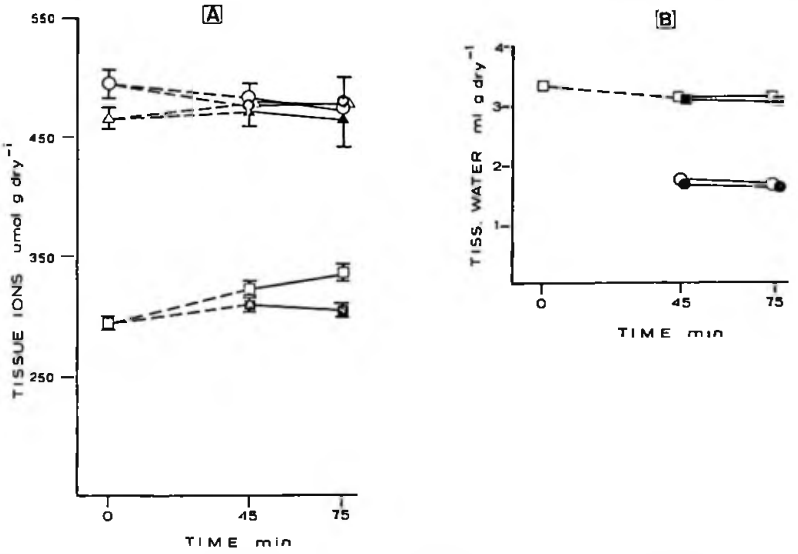


Figure 2. The effect of 5 x $10^{-4}$ M furosemide on tissue ions and water in untreated and cAMP/theophylline-treated slices of rectal gland. See Figure 1 for key to symbols.

chloride as well as their independent exit (alternative (i) above), then the addition of furosemide to block this entry should lead to a fall in tissue sodium and chloride levels. From the data presented in Figure 2, it is clear that no such decline in tissue sodium and chloride levels occurs and that in fact the addition of $2.5 \times 10^{-4}$ M furosemide produces no significant change in tissue ions in either control or cAMP-treated tissue.

It is concluded that the results of these preliminary experiments indicate that a major action of cAMP in the rectal gland is the stimulation of sodium entry into the cells and furthermore that this stimulated sodium uptake is largely independent of any mechanism of chloride uptake. We suggest therefore that, contrary to current models, any furosemide-inhibited coupled sodium-chloride co-transport system can be of only minor significance in the overall mechanism of cAMP-stimulated secretion in the dogfish rectal gland.

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## OUABAIN-BINDING IN THE RECTAL GLAND OF *Squalus* - THE EFFECTS OF CYCLIC AMP, SODIUM AND FUROSEMIDE

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In slices of the rectal gland of the European dogfish, *Scyliorhinus canicula*, the presence of dibutyryl cyclic AMP (cAMP) and theophylline in the incubation saline produces a marked increase in ouabain binding (Shuttleworth and Thompson, J. exp. Zool. 206:297-302, 1978). However, attempts using both perfused preparations (Silva *et al.*, Bull. MDIBL 18:16-19, 1978) and tissue slices (Epstein, personal communication) have failed to demonstrate a similar increase in glands from *Squalus acanthias*. This apparent discrepancy was investigated with a view to determining whether it represented a species difference or was the result of a difference in the techniques employed.

Rectal gland tissue was taken, sliced and incubated in a manner essentially similar to that described previously (J. exp. Zool. 206:297-302, 1978). The chief differences were that the incubating temperature was 15°C and that the total concentration of ouabain in the incubation medium was $2.25 \times 10^{-6}$ M containing $^{3}H$ ouabain at 0.25 µCi $ml^{-1}$. Tissue samples were removed from the incubation medium, given three five-minute washes in ice-cold ouabain-free saline and blotted dry. Following over-night drying at 95°C, the samples were weighed, solubilized (Soluene, Packard) and the degree of ouabain binding determined by liquid scintillation spectrometry. All results were expressed as pmol ouabain bound per mg dry weight ( ± S.E.).

Preliminary experiments were run to determine the time required for complete labeling of available sites in the tissue. No significant increase in binding occurred beyond 3 to 4 hours, so an incubation time of 4 hours was used in all subsequent experiments. The results are presented in Table 1.

The degree of ouabain binding in tissue from *Squalus* incubated in normal dogfish Ringer was very similar to that obtained previously for *Scyliorhinus* rectal gland (18.3 ± 1.3 pmol mg $dry^{-1}$). Incubation in 0.05 mmole $l^{-1}$ cAMP and 0.25 mmol $l^{-1}$ theophylline produced an increase of approximately 19% in the degree of ouabain binding which was statistically significant ($t = 2.574$, $P < 0.05$). This increase is clearly very much less than the 87% increase found in *Scyliorhinus* but indicates that the difference between the two species in this respect is quantitative rather than qualitative.

An intriguing aspect of these experiments is the long time periods required for complete ouabain binding (2 hours in *Scyliorhinus*, 4 hours in *Squalus*) compared to the very rapid effects of ouabain on