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Effect of mechanical stress on the calcification pattern in regular echinoid skeletal plates

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Abstract: The effect of tensile mechanical stress on the calcification pattern of isolated test plates of the echinoid Tripneustes gratilla elatensis was examined, using a radioisotope. Interambulacral plates, stretched in the horizontal direction (adradial-interradial) calcified at higher rates than the vertically stretched, or non-stretched plates. The ratio between the calcification rates at the upper and lower sutural margins and those of the horizontally directed (v/h ratio) was also affected in the treated plates: Plates stretched at the horizontal direction showed lower v/h ratios than the vertically stretched or the non-stretched. These results suggest that in situ the test plates are subject to mechanical stresses - tension along the longitudinal sutures and compression at the horizontal sutures.

1 INTRODUCTION

The hypothesis that peripheral growth and calcification of regular echinoid plates are controlled by a biomechanical effect - enhanced by sutural tensile stress and inhibited by compressional stress (Moss & Meehan 1968, Raup 1968, Dafni 1980,1983a), has never been tested. Heatfield (1970) and Meikle et al.(1979) proposed methods by which isolated skeletal parts (echinoid spines, mammalian cranial bones) could be used in radioactive in vitro experiments, to test the effects of chemical agents and mechanical forces. These methods were applied to test the above hypothesis.

2 MATERIAL AND METHODS

Five homologuous square pieces, each ca 2.5cm^2, were cut with a rotating disc, from the different superambital interambulacra of the coronal test of a living T. gratilla elatensis Dafni(1983b)(Fig. 1A), and cultured in 0.5 liter well-aerated seawater, at room temperature (25±1^{0}C) and continuous light. The explants were attached to a stretching device (Meikle et al. 1979), comprising of a Teflon cylinder (ϕ 20mm), upon which two moveble pin-carrying half-cylinders moved, pulled apart by a 50-100g stain-

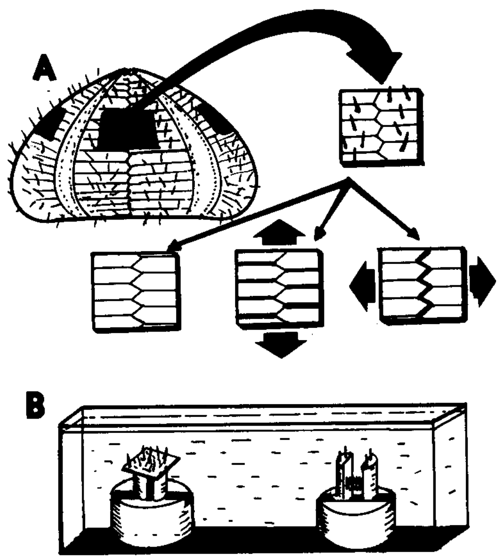

Fig. 1: Scheme of experimental procedure. A, skeletal squares removed from the superambital interambulacra and stretched in the horizontal direction (right), vertically (middle) or non-stretched. B, the stretching device, submerged in seawater; left - an explant is shown in stretched position.

Table 1. The effect of mechanical stretching of isolated T.g.elatensis test plates on (A) their calcification rates, and (B) on the ratio between vertical and horizontal sutural calcification rates (v/h), under different stretching conditions (vertical, horizontal stretching and non-stretched control). In parentheses - number of plates analysed for each treatment. The results were tested in a two-tailed Student's T-test.

A. Calcification rates ($\%.day^{-1}\pm SD\times10^3$)

Individual no.	Parts analysed	Direction of stretching			Comparison between treatments†		
		Horizontal	Vertical	Control	Hor-vert	Vert-cont	Hor-cont
y-23A	Whole plates	---	30±10 (17)	22±5 (18)	---	1.39*	---
y-9	Sutural edges	104±55 (10)	---	64±24 (13)	---	---	1.63*
y-12	" "	108±26 (20)	82±21 (22)	74±11 (9)	1.32***	1.1(-)	1.46***
y-17	" "	64±14 (18)	68±13 (19)	---	0.94(-)	---	---
y-20	" "	87±47 (15)	49±26 (20)	42±20 (9)	1.77***	1.17(-)	2.07*

B. Effect on v/h ratio

Individual no.	Parts analysed	Horizontal	Vertical	Control	Hor-vert	Vert-cont	Hor-cont
y-9	Sutural edges	0.47±.19 (10)	---	1.11±.64 (13)	---	---	0.42***
y-12	" "	0.71±.22 (20)	1.09±.26 (21)	1.20±.34 (9)	0.65***	0.91(-)	0.59***
y-17	" "	0.73±.25 (18)	1.17±.48 (19)	---	0.63***	---	---
y-20	" "	0.12±.11 (15)	0.26±.28 (20)	---	0.46(-)	---	---
y-23	" "	0.47±.16 (19)	0.73±.42 (30)	---	0.65*	---	---

† Level of significance: (-), N.S.; *,$p<0.05$;**,$p<0.01$; ***,$p<0.005$

less steel spring (Fig. 1B). The explants were stretched either in the vertical (aboral-adoral direction, or horizontally. An impaled specimen without stretching served as a control. After incubation in $^{45}CaCl_2$ seawater for 12h the explants were transferred to non-radioactive seawater for 12h and then cleaned in NaOCl (5%). Determination of ^{45}Ca activity in the entire plates or at their periphery, along the sutural margins, followed the procedure described by Dafni & Erez (1982), and divided by the specific radioactivity of the incubation medium to obtain the absolute calcification rates (in % accretion per day).

3 RESULTS AND DISCUSSION

The skeletal explants maintained their vitality for at least two days, during which spines and pedicellaria were active and continued to move slowly, and the latters' jaws opened and closed frequently. Later the tissue vitality usually deteriorated. The low calcification rates, obtained during the first 24h (5-10% of the in vivo rates), are the unavoidable results of the physiological stress caused by both, the physical injury - which is known to reduce temporarily the calcification rates (Heatfield 1970) - and the use of seawater as culture medium. Nevertheless, a consistent pattern was shown, different than the patterns that characterize intact urchins (Dafni & Erez 1982)(Table 1): Explants of the urchin y-23A, tested to find whether vertical stretching enhances overall calcification, when compared to the non-stretched, showed that this was indeed the case. The stretched plates calcification rates were 1.39 higher than the control (Table 1A).

Analysis of the sutural edges provided additional information, mainly because both, absolute sutural calcification and the ratio between vertical (at the upper and lower margins) and horizontal calcification (vertical margins), the v/h ratio, were compared (Table 1A & B).

The plates of individual no. y-9, stretched only in the horizontal direction, showed increased sutural calcification, by a factor of 1.6. In other explants, horizontal and vertical stretching were compared with the former yielding calcification rates 1.3-1.7 greater than the latter (Table 1A).

As to v/h ratio, there was a nonsignificant difference between the vertically stretched plates and the controls (Table 1B), whereas horizontal stretching reduced this ratio significantly. This was due to the increase of the interradial (zigzag) suture calcification rates. It is noteworthy, however, that the v/h ratio of non-stretched sutures in this experiment were appreciably higher than the in vivo ratios (ca 0.3-0.4) (Dafni & Erez, in prep). This may suggest that the mere extraction of plates from the tight-fitting in situ mosaic affected both calcification rates and differential patterns.

These results support the hypothesis that in vivo the plates are under mechanical confinement (Raup 1968), which is more pronounced in the vertically directed sutural edges, due to the mechanical activity of the ambulacral tubefeet, that pull the entire test towards the substratum, pressing the vertical plate columns in this direction (Dafni 1980). Extracting the plates probably relieves this compression, causing increased v/h ratios even without the application of vertical stretching. Horizontal stretching possibly simulates the in situ tensile stresses in this direction, due to the expansion of the entire test (Moss & Meehan 1968) and to the spatial arrangement of the intestinal mesenterial threads (Dafni & Erez 1982), increasing sutural calcification and lowering v/h ratio.

It has been pointed out (Dafni, MS) that sutural gaps show often in rapidly growing sutures, and that skeletal trabeculae, that grow in these gaps, are abnormally long and slender. It seems logical that tensile stress, which pulls the plates apart, cause these gaps' formation, thus stimulating growth and calcification at the sutures, whereas compression goes together with the plates confinement.

Physical confinement is known to affect the growth and morphology of various cell aggregations and those of colonial organisms (Thompson 1942, Raup 1968, Stevens 1974). In the echinoid test stresses act in a more differential way, and a certain plate may be subjected to compression in one direction and tension in the other. This pattern may change appreciably as the plate shifts along the vertical column, during its ontogeny. It is also possible that mechanical "stress balance" is the main controlling factor that determines echinoid test morphology (Dafni MS).

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