FURROWING IN THE PRESENCE OF CAFFEINE-INDUCED MONASTERS IN SAND DOLLAR (*ECHINARACHNIUS PARMA*) EGGS

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Spindles in fertilized echinoderm eggs fail to develop in the presence of 10 mM caffeine. The centrosomes do not separate and the normal mitotic configuration of chromosomes is absent. Only one irregular aster structure forms and, although the egg contour becomes irregular at cleavage time, no furrow forms (Harris, *Developmental Biology,* 96:F277-F284,1983).The purpose of this investigation was to elucidate the causes of cleavage failure in the presence of caffeine.

Fertilized sand dollar eggs were immersed in 10mM caffeine in filtered sea water and then bisected with glass hooks or needles within 20 min. At the anticipated cleavage time the surface contours of the nucleated halves became irregular but the enucleated halves remained smooth and spherical. These results indicate that the formation of surface irregularities requires the presence of both caffeine and mitotic apparatus (MA) material. Irregularity formation by direct action of caffeine on the egg cortex is unlikely.

When caffeine-treated cells were constricted with glass hooks so that the two nearly spherical halves were connected by a 27 μm neck, the MA material was localized in one of the halves. Cytoplasm flowed from the nucleated half to the enucleated half and the MA material was also transported to the previously enucleated cell half. After nuclear relocation,the direction of cytoplasmic flow reversed. These results were similar to those obtained when the same manipulation was performed on untreated sand dollar eggs (Rappaport and Rappaport,*J. Exp. Zool.* .247:92-98,1988), although the flow began later in the mitotic cycle and the number of flow reversals was reduced. They indicate that MA material can induce cortical contraction in the presence of caffeine concentrations that block normal cleavage.

Caffeine-treated cells were reshaped into cylinders by confinement in transparent silicone rubber capillaries. In cylindrical cells, furrows formed perpendicular to the cell long axis in the plane of the center of the mass of MA material. Furrows were delayed; most furrows were completed but some regressed. These preparations also revealed that the form of the caffeine-treated MA material is variable. The results resembled those that follow when single asters are confined in otherwise normal cylindrical cells (Rappaport and Rappaport, *J. Exp. Zool.* 235: 217-226, 1985). They indicate that caffeine-treated cells can form cleavage furrows when they are reshaped into cylinders.

Although caffeine has non-specific effects that sometimes result in delay and regression of furrows, the results of this investigation suggest that the failure of furrowing that accompanies caffeine treatment may be due in large part to the fact that caffeine changes the overall shape of the MA from an elipsoid to a sphere.The effect of the MA on the cortex is distance-related. When the MA is reshaped into a sphere and confined within a spherical cortex the distance from the MA to the cortex is uniform throughout the cell. In this circumstance it is unable to induce the normally belt-shaped region of enhanced cortical contractility that constitutes the furrow.