REVERSING FLOW IN CONSTRICTED SAND DOLLAR (*ECHINARACHNIUS PARMA*) EGGS AND BLASTOMERES

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Animal cell division is caused by the contraction of a temporary narrow equatorial ring of specialized surface material that forms under the influence of the mitotic apparatus (MA). The mechanism of contractile ring formation is unknown, and the purpose of this investigation was to learn more of the way in which the MA affects the cell surface. Previous work involving artificially constricted eggs demonstrated that the surface closest to the MA contracts, forcing cytoplasm out of the nucleated region, beginning 26 min before the first cleavage. When the flow carries the MA into the previously unnucleated region, the direction of flow usually reverses. During the 90 min period between fertilization and first cleavage, events of syngamy as well as mitosis occur, and, in order to avoid syngamy, the surface contractile activity of artificially constricted blastomeres between the first and second cleavage was observed.

Jelly membranes and hyaline layers were removed from eggs by glycine treatment. Blastomeres (n=31) were separated immediately after the first cleavage and constricted (as nearly as possible) in the plane of the geometrical center to a diameter of 22-27 μm. The nucleus was displaced to one side of the constriction. In all cases, cytoplasm flowed out of the nucleated region. Flow was not confined to a limited part of the intercleavage period, as instances were observed from the time of constriction to 4 min before the anticipated time of cleavage, when the division mechanism for the subsequent cleavage is irreversibly established. It seems likely that the flow that occured immediately after constriction was caused by the parts of the first MA that normally segregate into each blastomere. Subsequently, flow must have been associated with the stages of formation and function of the second MA. In this geometrical circumstance, nuclear material appears capable of causing surface contraction during interphase as well as during mitotic stages.

The characteristic radiate structure of the MA is based upon microtubules. Agents, such as Colcemid, that block microtubule assembly before the division mechanism is established also block cleavage. Cleavage of spherical eggs immersed in 5 μm Colcemid 50 min after fertilization is completely blocked, but artificially constricted eggs exhibit cytoplasmic flow in the same concentration. In 10 μM Colcemid, 10 of 14 constricted eggs showed cytoplasmic flow. In both concentrations, the radiate structure of the MA is completely abolished. In treated constricted eggs, the flow began later than in untreated eggs, and it appeared to be somewhat weaker.

It appears that the activity of the MA that results in surface contraction is not dependent upon the normal microtubular configuration *per se*. The normal radiate structure of the MA may, however, facilitate the activity and insure a degree of geometrical precision during furrow establishment.
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