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LIPID METABOLISM IN DIVIDING SAND DOLLAR EGGS

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Since remarkable changes occur in the E. parma egg membrane from fertilization, through division and differentiation, a study was planned to investigate the lipid metabolism in fertilized and unfertilized E. parma eggs and membranes. It was hopes that some correlation could be obtained among the physical and chemical changes that occur in the membrane and the changes in shape, structure, and permeability that occur in dividing cells.

A method was devised to fracture E. parma eggs and to separate cell membranes from intracellular material. Egg membranes from fertilized eggs that were incubated in sea water at 16-17° for 2.5 hours showed a twofold increase in the total extractable lipid when compared to unfertilized eggs treated in the same manner. During this time more than 90 percent of the fertilized eggs had reached the four-cell level of development. This was in agreement with the concept that the cell membrane was being newly synthesized.

In order to follow the metabolic events that were involved in membrane synthesis in the dividing cells, experiments were designed using exogenous tracer ^{14}C-labeled palmitic acid to follow incorporation into those lipids being synthesized in whole cells. In unfertilized eggs incubated in filtered sea water at 16° C there was a slow but constant, measurable uptake of palmitic acid into both neutral lipids and phospholipids over a two-hour period. This incorporation was principally into triglyceride, phosphatidyl choline, phosphatidyl ethanolamine, and several unidentified polar lipids and lysophosphatides. In fertilized eggs the picture was markedly different. The incorporation into neutral lipids was generally increased with more label being incorporated into triglyceride and higher levels of ^{14}C-palmitic label in diglyceride and monoglyceride. Probably of more significance was the fact that incorporation was constant in the fertilized eggs until 70 to 80 min after fertilization when the incorporation rate into triglyceride suddenly doubled. Phospholipids in the fertilized egg revealed a similar but more exaggerated picture. From 70 to 80 min after fertilization, incorporation of radioactive fatty acid into phosphatidyl ethanolamine, phosphatidyl choline, and lysophosphatides was at least tripled.

Since the fertilized eggs underwent the first cleavage approximately 96 min after fertilization, the increased incorporation of fatty acid commencing at the 70 to 80 min period or mid-mitotic stage indicated the triggering of some processes prior to the actual cell cleavage. It remained to be shown, however, what portion of the newly formed lipids were in the membranes.

To eliminate permeability problems of the tracer fatty acid a study was undertaken using cell homogenates. Fertilized and unfertilized E. parma eggs were incubated in sea water but without fatty acid. At predetermined times the developing eggs were centrifuged carefully; and the packed eggs were homogenized in Tris chloride buffer, pH 7.37 with K palmitate-^{14}C. The homogenates were incubated at 25° for 15 min and were then denatured and extracted with solvents after which the extractable lipids were analyzed.

In the unfertilized homogenates, radioactivity was uniformly incorporated into triglyceride by all samples but insignificant quantities were incorporated into other neutral lipids. There was incorporation of label into phosphatidyl choline, phosphatidyl serine, phosphatidyl inositol,

and phosphatidyl ethanolamine indicating a general activity of the phospholipid pathways. Homogenates from fertilized eggs differed in several aspects. There was a more active conversion into triglyceride and there was a distinct increase of the incorporation of lipid into diglyceride 70 to 80 min after fertilization. Incorporation of the fatty acid into the phospholipids was similar to the unfertilized samples except for phosphatidyl ethanolamine which also showed increased radioactivity at the 70 to 80 minute stage.

It is expected that continued studies on the biochemical events occurring in the early and mid-mitotic stages will provide information for understanding the mechanism of cytokinesis.

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STUDIES OF ORGANIC ACID, SUGAR, AND AMINO ACID TRANSPORT IN ISOLATED PERFUSED FLOUNDER TUBULES

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Using methods previously described (The Bulletin 6:7, 1966) single proximal renal tubules of the flounder Pseudopleuronectes americanus were perfused in vitro. Steady state concentration of transported substrates were determined in the external bathing solution, tubule fluid, and renal cells.

In 10 experiments the addition of PAH-H^3 (2×10^{-5} M) to the external bath, resulted in secretion of PAH into the lumen at a rate (63.8×10^{-14} M min^{-1} mm^{-1} tubule length) which is approximately equal to that previously observed for the same concentration os iodopyracet (56×10^{-14}). The concentration of PAH in the tubule fluid was 21.6 times as great as in the bath, similar to the ratio for iodopyracet (17.2). In contrast, however, the concentration of PAH in the cells was much lower (12.7 times that in the bath) than for iodopyracet (133). Thus, the concentration profile of organic acids during transport by flounder tubules varies markedly, depending on the compound studied. For chlorphenol red the concentration is several orders of magnitude greater in the tubule fluid than in the cells, for PAH it is only twice as great, but for iodopyracet it ís lower in tubule fluid than in cells. These results are most simply explained by differences in the handling of various organic acids by two active transport mechanisms, one at the luminal and the other at the peritubular cell membrane. However, it is also possible that a portion of intracellular organic acid is bound and that this fraction differs depending on the compound studied.

When inulin-C^{14} was placed in the external bath very little appeared in the perfusion fluid (final concentration in the perfusion fluid in 3 experiments .04 times that in the bath) indicating low permeability to inulin. Also, when inulin-C^{14} was placed in the perfusion fluid there was little change in its concentration (ratio of .90 collected/perfused in 9 experiments) indicating little net fluid movement.

When PAH-H^3 (3×10^{-4} M) was placed in the perfusion fluid, there was some loss from the tubule lumen (mean PAH/inulin ratio in the collected fluid was .79 in 7 experiments). The PAH permeability measured by this efflux from the lumen is small, however, compared to the rapid