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THE VELOCITY OF PROTOPLASMIC STREAMING IN FORAMINIFERA

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The Foraminifera are amoebae which are characterized by very long slender pseudopods (often called reticulopods) which branch and anastomose to form a complex network. The smallest reticulopods have a diameter of about a micron and are composed of only two streams of protoplasm flowing in opposite directions. There is no evidence for any stationary material in the reticulopods of healthy individuals (Jahn and Rinaldi, Biol. Bull. 117, 100, 1959). The larger reticulopods seem to be composed of bundles of the simple ones. It has been noted that when a reticulopod is extending or contracting one stream moves faster and the other slower in such a way that the relative velocity of the two streams is unchanged (Jahn and Bovee, Physiol. Rev. 49, 813, 1969). Precise measurements of the relative velocities have not been made and no studies have been made of the effect on this velocity of changing physiological conditions. It would be an important step toward the understanding of the mechanism of biological motion if it should turn out that this velocity is always exactly the same or that it changes with environmental conditions in a reproducible way.

The streaming velocities were studied for about a dozen different species of foraminifera. These included both free and attached forms and both arenaceous and calcareous types. The animals were collected from the surface of sandy mud at low tide in Emery Cove. Much of the work was done with one species of *Quinquiloculina* which was abundant and easy to recognize in the field. The streaming was observed under a cover glass using 400X phase contrast and timed with a stop watch. The optical system allowed resolution of all but the smallest of the reticulopods. Some of the experiments with changing physiological conditions were done with several different species under the same cover glass. In every case all species reacted identically.

The relative streaming velocity in the reticulopods of healthy foraminifera was found to be 18 ± 15 percent μm/sec. Even when various chemical, thermal, and electrical stresses were impressed on the animals the velocity remained unchanged. Parts of the net stopped streaming and those parts that still showed streaming contained stationary granules. It seemed likely that the slowly-moving granules in such animals were being dragged through motionless cytoplasm by currents of moving cytoplasm. The chemical stresses that did not affect the protoplasmic streaming included: replacing half of the Na^+ with an equivalent amount of Mg^{++}, replacing one third of the Na^+ with K^+, dilution of sea water to 90 percent normal salinity, 10^{-3} molar $NaSO_4$, and 10^{-4} molar 2-4-dinitrophenol. Lowering the pH did not change the relative streaming velocity but did change the appearance of the reticulopods. They became shorter and more active. At a pH of 5.3 they looked almost like flagella. The time scale of their motions was such that a reticulopod could bend into a U shape and straighten again in less than a second. When the pH was restored to normal the animals regained their normal appearance.

The relative streaming velocity was constant over a temperature range of 13°C to 24°C (normal sea water was 15°C). When the laboratory was above 24°C the animals would not come out of their shells. When they were cooled from 22°C to 4°C in 15 minutes they died. With slow cooling at about 12°C the streaming began to be slower with increasing amounts of stationary material. It is not clear how to interpret the reduced speed. The animals were observed to survive for a number of days at 4°C. It would be interesting to study the animals from the same location in winter when they are acclimated to a low temperature.

No change in relative streaming velocity was caused by electric currents of magnitude just under that which made the animals withdraw into their shells. Magnetic field up to 50 gauss had no effect on the streaming velocity.

If the relative streaming velocity is indeed an invariant quantity, as is suggested by these experiments, it must be a manifestation of some fundamental process. To predict how the same process would manifest itself in other systems that are structurally different, it is necessary to have a model. A model that predicts an invariant relative streaming velocity from quantum mechanical traveling waves on long thin molecules (like F-actin) will be described elsewhere. An application of this model to gliding diatoms predicts that they would have a velocity of 18 μm/sec when they are moving at a steady rate. This prediction was verified for several different kinds of diatoms that were collected along with the foraminifera.

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THE TIME OF APPEARANCE OF SPECIFIC PROTEINS IN EMBRYOGENESIS

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Protein extracts were prepared from a large series of early stages of zygotes and embryos of *Fundulus heteroclitus* as part of an extended program in the development of a catalogue of embryonal and adult proteins and their time of appearance in relation to morphogenesis.

At any particular stage aliquots of embryos (approx. 500) were selected and pulsed with ^{3}H-valine or ^{3}H amino acid mixture (20μCi/ml) for eight hours. Following the pulse the embryos were extensively washed and permitted to develop further.

At Stage 18-21 the embryos were homogenized in 0.15M sucrose and 0.01M Tris, pH 7.6 (2:1 v/v) in a teflon-glass homogenizing system. The chorions were removed by mild centrifugation and the crude homogenate was then further centrifuged at 105,000 x g for 90 minutes.

Following this the supernatant was collected and placed in SDS buffer (0.2 percent sodium dodecyl sulfate, 0.02 percent mercaptoethanol and 0.01M Tris at pH 7.0) in a v/v of 1 and heated in boiling water for 15 minutes.

The homogenates were dialyzed overnight against 0.2 percent SDS and 0.01 percent mercaptoethanol and 0.01M tris.

Samples were removed for the assay of bulk protein separation using SDS acrylamide gel electrophoresis. The remaining protein was stored at –40°C for further study.

Comparison of sample runs stained with coomassie blue indicated that the bulk proteins so displayed were remarkably similar in electrophoretic pattern. The subtle changes known to be occurring in embryogenesis are hidden within this pattern and are demonstrable only by isotopic incorporation patterns. These are currently under study using the frozen samples. Details of the technique and preliminary results are to be found in Schwartz, R.J. and Wilde, C.E., Jr., Nature 1973 (in press).
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