XLVIII. ARTIFICIAL PARTHENO-GENESIS IN SEA URCHINS.

A NOTE ON AN IMPROVED TECHNIQUE.

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WORKING at the Marine Biological Laboratory, Plymouth, at Easter, 1921, the writer followed Shearer and Lloyd [1913] in their modification of Loeb's [1913] technique for the induction of artificial partheno-genesis in sea urchin (*Echinus esculentus*) eggs.

The eggs were shaken into a bowl of fresh "outside" sea water and, if found of the right maturity, were transferred in turn by pipetting into a series of bowls containing the following solutions in the order given, and for the times stated:

- (1) 0.3 cc. *N*/10 butyric acid + 50 cc. sea water. 1.5 minutes.
- (2) Sea water. Wash
- (3) " " } 15 minutes.
- (4) 0.2 cc. *N*/10 NaOH + 50 cc. sea water. 6 minutes.
- (5) Sea water. Wash.
- (6) 8.0 cc. 2.5 *M* NaCl + 50 cc. sea water. 45–60 minutes; or 25–30 minutes.
- (7) Sea water. Wash.
- (8) Final transfer into normal sea water.

These manipulations certainly resulted in segmentation of the eggs, and in the production of swimming blastulae. But the yield varied enormously from experiment to experiment; and, where it was necessary for other purposes to look for mass production, the above method, at any rate in the writer's hands, failed to give consistently good results. Conditions inherent in the egg could not account for the differences. For, specimens of eggs from the same batch treated in the same way at the same time displayed widely differing mortality. Plainly, the discrepancy crept in from outside. Errors in the composition of the various fluids would offer a sufficient explanation.

The pipette method of transfer seemed the most likely source of such error. At each transfer at least 50 %—more often 90 %—of the eggs remain behind in the bowl. On the former generous scale of diminishing returns, where there are at least eight transfers, the last bowl will contain $\frac{1}{2^8}$ ($\frac{1}{256}$ th) the number of eggs treated in the first bowl. To obtain any visible number of eggs in the end bowl, it was found that at least 3.0 cc. of sea water containing a thick emulsion of eggs had to be used to commence with.

Now, of what use is it to prepare, meticulously, the exactly proportioned solution containing *e.g.* 0.2 cc. *N*/10 NaOH in 50.0 cc. sea water when an immediate contamination of 3.0 cc., that is 6.0 % is involved in the mere transfer? More often, however, 6.0 cc. or even 9.0 cc. (18 % error) is transferred, or else it is quite impossible to pick out the eggs in the eighth bowl. One has to search for the number of eggs in $\frac{1}{256}$ of 3.0 cc. scattered in a solution of 50.0 cc. sea water. Plainly, at least 6 % error, and more often 12 % to 18 % error, is involved in each transfer. Here then, we may say we have discovered the source of the differing end-results.

The remedy is simple. The experiment must be so arranged that the eggs remain in the same vessel throughout; the changes of medium being effected by drawing off the fluid.

A deep funnel, with a securely fitted filter answers the purpose. The writer adapted the funnel of a Cona coffee still; the filter pad is firmly applied to the bottom of the funnel by means of a long wire passing through the limb of the funnel. A screw at the bottom of the wire serves to tighten the filter pad as much as desired.

The filter pad itself consists of a perforated metal or porcelain disc covered with several layers of fine muslin sewn into the disc. Layers of fine filter paper may be super-added if it is found that the eggs pass through.

A piece of rubber tubing, supplied with a tap, is fitted to the end of the limb, and in this way it is possible to permit the action of a solution for any length of time before withdrawing. Draining off can be effected much more rapidly by the judicious and gentle use of a vacuum water pump, to which the apparatus can be easily attached by rubber tubing. A further advantage in this method is that the eggs can be easily washed any number of times by flushing the apparatus through with sea water.

In conclusion, it may be said that the method is suitable not only for the partheno-genetic treatment of eggs, but for all experiments in which eggs, fertilised or unfertilised, are to be exposed to the action of various fluids.

SUMMARY.

(1) The "pipette" method of transferring eggs from one bowl to another is laborious, time consuming and involves a minimum error of 6 %; more often 12%-18 %.

(2) The new "funnel" method described is easily adapted to laboratory material, involving the use of a flat-bottomed funnel with perforated plate, muslin and filter paper. A ready made efficient substitute is indicated in the funnel of the "Cona" coffee brewing apparatus.

(3) The method obviates the errors of transfer; it is simple, rapid and involves no eye-strain.

REFERENCES.

- Loeb, J. (1913). *Artificial Partheno-genesis and Fertilisation*, Chicago.
 Shearer and Lloyd (1913). *Quart. J. Mic. Sci.* 58, 523.