## Separation of Micromeres of the 16-Cell Stage of the Sea Urchin *Paracentrotus lividus*

A comprehensive study of the chemical and physiological properties of cells occurring naturally in suspensions together with other cells, or connected to other cells, is possible only after their separation in sufficiently great amounts. As a first step towards such a study of the three kinds of blastomeres in the sea urchin 16-cell stage, the macro-, meso- und micromeres, we have worked out the isolation of micromeres using a counter-streaming centrifuge, the principle of which was sketched by LINDAHL[1]. Among the three kinds of blastomeres of this stage, the micromeres deserve special interest. They represent the most vegetal material of the egg and exercise a decisive influence upon the development of other parts of the embryo[2]. Besides, they show several properties indicating a state of cytoplasm different from that of the other blastomeres[3,4].

The centrifuge in question makes possible the separation of differently sized particles having equal density. A higher density of the larger particles than of the smaller ones favours the separation, whereas the reverse counteracts it. However, the three kinds of blastomeres of the 16-cell stage of *Paracentrotus lividus* do not differ significantly as to their density[5].

The egg suspensions, prepared in the usual way, were fertilized, and the eggs freed from the fertilization membranes by sucking them through bolting silk at a suitable rate[6]. Then the membranes and the jelly coats were washed away in a hand-driven centrifuge and the diluted suspensions gently shaken in rectangular troughs placed on a shaking machine[4]. When the first sixteen cell stages appeared, the eggs were twice washed with $Ca^{++}$-free sea-water and finally suspended in this medium containing $2 \cdot 10^{-3}$ M of colchicine (cf. BEAMS and EVANS[7]) to stop further cleavage and losen the blastomeres from each other. The latter purpose was forwarded by repeatedly pouring the suspension slowly from one vessel into another. Cells still sticking together were cautiously centrifuged down in the hand-driven centrifuge, and the supernatant suspension was introduced in the counter-streaming centrifuge, in which sea-water was used as a medium. The separation was carried on for 56 minutes[8] with 900 R.P.M. ($n$)[9] and a streaming velocity ($V$) of 0·22 ml/sec. The greatest diameter ($R$) of the separation chamber was 2·4 cm, and the distance ($L$) between this and the theoretical point of the chamber cone was 18·0 cm. The chamber was mounted so, that this theoretical point was 24·0 ($Z$) cm from the centre of rotation.

The greatest volume of densely packed micromeres obtained in an experiment was about 0·07 ml, but this volume could easily have been multiplied several times by injecting a greater amount of cell suspension into the counter-streaming centrifuge. However, the yield of micromeres was not 100%, single ones remaining in the fraction composed of macro- and mesomeres. This will be overcome by carrying on the separation for a longer time and using higher streaming velocities.

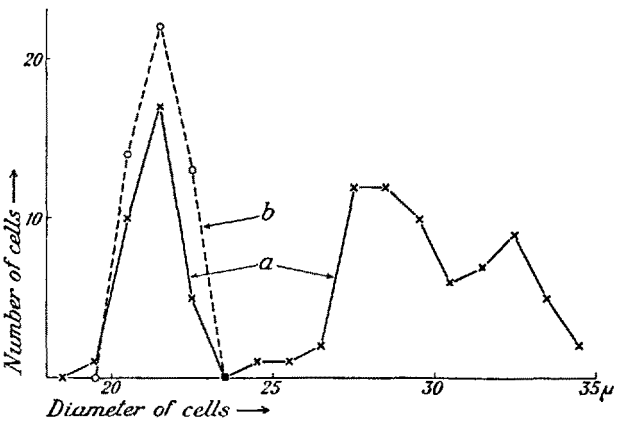


Fig. 1. – Ordinate: Numbers of blastomeres. Abscissa: Diameters of blastomeres in $Ca^{++}$-free sea water measured by the aid of an ocular screw micrometer (a scale unity equal to 0·38 $\mu$ at the magnification used). *a* mixed 16-cell stage blastomeres, *b* separated micromeres.

In the above-mentioned experiment giving the maximum volume of packed micromeres, the diameters of 50 separated micromeres were measured and compared with the diameters of sixteen cell-stage blastomeres (Fig. 1). Obviously there are only micromeres among these measured 50 cells, and the examination of several hundreds of separated cells did not reveal any cell larger than the largest micromeres[1].

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*Zusammenfassung*

Es wird eine Methode zur Herstellung von reinen Suspensionen der Mikromeren des 16-Zellen-Stadiums von *Paracentrotus lividus* (Seeigel) ausgearbeitet, wobei die von LINDAHL[2] konstruierte Gegenstromzentrifuge Verwendung findet.

[1] These experiments were carried out at the Zoological Station at Naples. We express our hearty thanks to Prof. R. DOHRN, the director of the station, and his staff, for their kind support of our work.

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[6] P. E. LINDAHL and J. LUNDIN, Science *108*, 481 (1949).

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[8] The time of separation was limited by certain construction details, which will be altered in a near future.

[9] The letters in brackets refer to the formula by LINDAHL (1948) for the calculation of $r_{min}$, the critical radius diameter separating two fractions of particles.

## Blastokinesis and Embryonic Development in a Phasmid

No conclusive information is as yet available on the mechanism and function of blastokinetic movements of insect embryos. Opinions differ even regarding the necessity of these revolutions for the completion of normal embryogenesis[1]. During a recent study of the embryonic development of *Bacillus libanicus* certain observations concerning these problems were made.

In this species blastokinesis consists of three main, separate movements. The first movement begins while the embryo is still in the unsegmented germ-disc stage, situated on the ventral surface of the egg near the posterior pole; the protocephalic region is directed towards the anterior pole of the egg. By gradually

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