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.

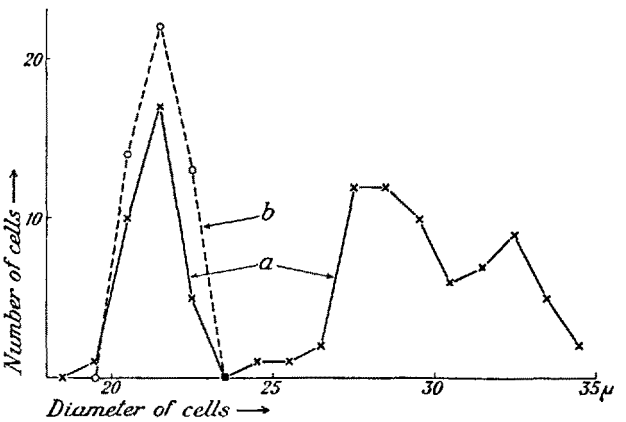

Fig. 1. – Ordinate: Numbers of blastomeres. Abscissa: Diameters of blastomeres in Ca^{++}-free sea water measured by the aid of an ocular screw micrometer (a scale unity equal to 0·38 μ at the magnification used). *a* mixed 16-cell stage blastomeres, *b* separated micromeres.

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.

[2] P. E. LINDAHL, Nature *161*, 648 (1948).

[1] P. E. LINDAHL, Nature *161*, 648 (1948).

[2] S. HÖRSTADIUS, Biol. Rev. *14*, 132 (1939).

[3] J. RUNNSTRÖM, Protoplasma *4*, 388 and *5*, 201 (1928).

[4] P. E. LINDAHL, Acta zool. *17*, 179 (1936).

[5] J. LUNDIN, unpublished experiments.

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

[7] H. W. BEAMS and T. C. EVANS, Biol. Bull. *77*, 328 (1939).

[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.

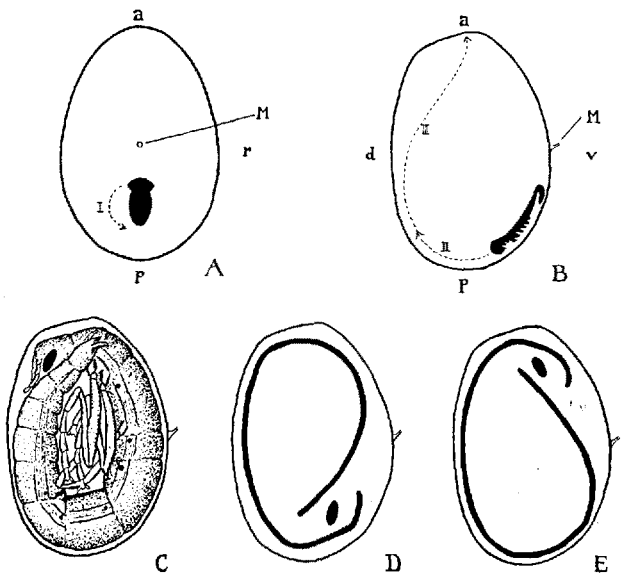
Blastokinesis and Embryonic Development in a Phasmid

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

[1] F. SEIDEL, Arch. Entw. Mech. Org. *2*, 322 (1929). – M. TIRELLI, Zool. Jb. *49*, 59 (1931). – E. H. SLIFER, Biol. Zbl. *52*, 223 (1932).

rotating to its right, around a central transverse axis, the embryo moves in an anticlockwise direction on the ventral surface of the egg (Fig.1,*A*). The original orientation of the embryo is thus changed by 180°, the originally cephalic end now being directed towards the posterior pole of the egg. This first phase of blastokinesis lasts for 4 to 5 days and is accompanied by marked growth and differentiation. Six days later the second phase begins, which consists of a meridional movement around the



A Semi-schematic representation of the ventral surface of egg with germ-disc, before the beginning of blastokinesis; I direction of first phase movement; M micropyle.
B Side-view of egg and embryo at the end of first stage movement; *II* direction of second-stage movement; III direction of third-stage movement.
C Side-view of normally situated, fully developed embryo.
D, *E* Position of fully inverted embryo due to suppression of the second phase (*D*), and of partly inverted embryo due to suppression of the first phase of blastokinesis (*E*).

posterior pole, shifting the embryo from the ventral towards the dorsal surface of the egg; its head is now again directed towards the anterior pole (Fig.1,*B*). This phase lasts for about 25 to 30 hours, during which there is only slight additional growth. The third phase, which begins 1 to 3 days after the termination of the second, is marked by accelerated growth and consequently by increased absorption of the extraembryonal yolk. Facing the dorsal surface of the egg the embryo moves anteriorly until its head reaches the opercular cap (Fig. 1, *C*). In this final position its development is completed and the embryo enters into diapause terminated by hatching.

The third phase movements apparently result from the growth and elongation of the embryo and the decrease of the extraembryonal yolk. At the anterior part of the egg the yolk is used up most rapidly and the growing embryo passively expands in this direction.

The second phase movements were studied through direct observation of living embryos. This was carried out by stripping eggs at this stage of their opaque exochorions and keeping them in a moist chamber. This procedure caused no immediate serious damage as the remaining transparent envelopes maintained the egg in a condition enabling the embryo to continue its development for some time. The movements of the embryo thus observed consisted of alternating periodic jerks to its left and right, each motion advancing it slightly forwards along a zigzag line. The second phase movement is thus actually a summation of several separate motions of the embryo. SLIFER[1], who observed a similar process in *Melanoplus*, tried to locate the mechanism of these movements in contractions of certain spindle-shaped cells, probably precursors of the abdominal muscles. Similar cells are also present at this stage in *B. libanicus* and their position supports SLIFER'S assumption.

The second phase movements could be prevented by transferring eggs at the end of the first phase to a temperature of 10° C for 8 to 10 days. Many eggs treated in this way resumed their development when returned to optimal conditions and the embryos reached full differentiation. But their orientation, compared with that of normal embryos was found to be fully inverted (Fig. 1, *D*), which indicates that after resumption of development no further blastokinetic movements occurred, and the embryo remained in the pre-second phase position. No structural malformations were observed in such embryos, so that, in spite of their inverted position, they apparently developed normally, although they were, of course, unable to hatch.

The first phase movements could not be prevented by low temperatures; those of the embryos which recovered from the treatment, rotated. The early stage at which these movements start suggested a possible association between their mechanism and the structural organization of the egg. To probe this possibility centrifugations of eggs were carried out. It was found that 4-day eggs centrifugated at 1,500 r. p.m. for 10 minutes (which caused no visible stratification) completed their development and that in 32 out of 50 such eggs no blastokinesis had occurred. This could be judged by the orientation of the fully developed embryos which, on examination, was found identical with that of the initial germ disc (Fig.1,*E*). As the examination did not damage the embryos (they were observed through the opercular aperture, the lid being easily replaceable) many of them hatched, thereby demonstrating that they were normal. It is noteworthy that the above condition was not found to occur in any of the more than a thousand normal untreated eggs examined.

It appears therefore, that in *B. libanicus* embryonic development may proceed in the absence of the typical blastokinetic movements. The prevention of the first phase movements by slight centrifugation of the egg, points to its possible relation to changes in the spatial organization or orientation of the egg components. The temperature sensitivity and the nature of the second phase movements support the assumption that they result, at least in part, from contractions of the embryo. The third phase movements are apparently due to the passive expansion of the growing embryo and, as such, may also occur in the absence of the two preceding phases.

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Zusammenfassung

Die Blastokinese des Embryos der Stabheuschrecke *B. libanicus* besteht aus drei getrennten Bewegungen. Hinweise auf den Mechanismus der einzelnen Bewegungen ergeben sich aus den Resultaten der Zentrifugierung und Kühlung der Eier wie auch aus den histologischen Untersuchungen der Embryonen selbst. Es erwies sich als möglich, die Blastokinese experimentell vollkommen zu verhindern, anscheinend ohne jede Schädigung der sich entwickelnden Embryonen, welche ohne Störung schlüpften und sich normal weiter entwickelten.

[1] E.H. SLIFER, Biol. Zbl. *52*, 223 (1932).