Manipulation of Cleaving Mammalian Embryo with Special Reference to a Time-Lapse Cinematographic Analysis of Centrifuged and Fused Mouse Eggs

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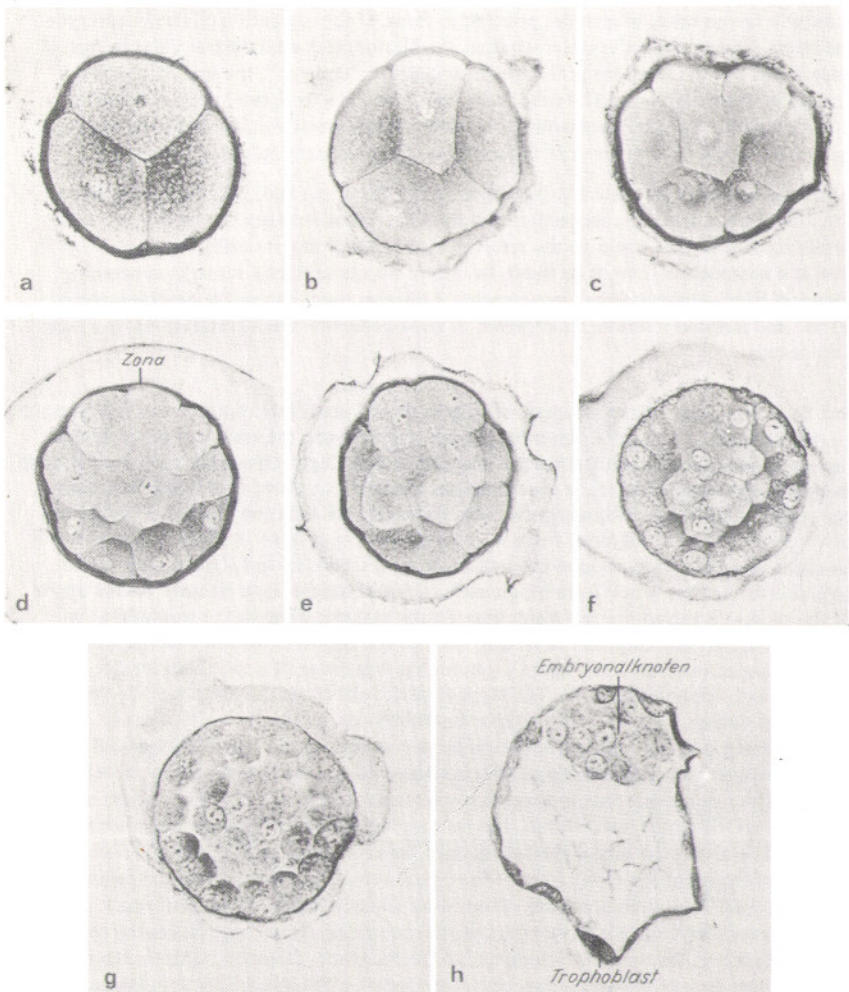
Discussion

Denker: Your discussion of the concepts of epigenesis and predifferentiation encourages me to make a short remark. There are differences in each case between the results of physiological experiments demonstrating regulative capacities and morphological observations demonstrating cell lineage. These morphological or histochemical observations show, that in the very early cleavage stages of the rabbit two categories of blastomeres are clearly separated. I should like to show you some figures. All these fotos were taken from eggs fixed by formol-alcohol-acetic acid, as *Dalcq* and his associates have used, then paraffin sectioned and stained with bromphenol blue. On the first foto (figure h), an early rabbit blastocyst is to be seen. At the upper part of the picture is the embryonic knot, only faintly stained, whereas the trophoblast is stained a little more. The following fotos lead back to the earlier developmental stages (the figures are arranged inversely). On figure g you see the beginning of a split between the embryonic knot and trophoblastic cells. The trophoblastic cells are in the lower part of the slide and are stained darker. Figure f shows a three day rabbit egg, a morula, without cavity. You see a clear difference in stain uptake between the two categories of cells. The inner cell mass is lightly stained, and a peripheral cap of cells is stained darker, with a maximum of stain uptake in that part of the cytoplasm that is directed to the center of the egg. On figure d you see an egg at an earlier stage, 63 hours post coitum, and here again you can distinguish two groups of blastomeres. The one at the top of the foto is only faintly stained and the other is stained darker (the lower part of the foto). I am of the opinion that the dark cells are the presumptive trophoblastic cells. They show the same cytoplasmic characteristics I have mentioned: They have the maximum stain uptake in that part of the cytoplasm, which approaches the center of the egg. Furthermore, the nuclei are elongated in the tangential direction, whereas the lighter cells, in the upper part of the slide, show nuclei elongated in the radial direction. On a section

of an other egg, 63 hours p. c., (figure e), you see the dark cells elongated tangentially. The light cells are arranged in a group separately forming a kind of a knot. The next foto (figure c) shows an egg 40 hours p. c. It has about 12 cells. Again two groups of blastomeres are distinguishable: one stained lightly and one stained darker. The darker cells could be the presumptive trophoblastic cells. At an even earlier stage, the 8 cell stage (figure b), one cell is elongated radially, and the other, which are stained darker, are elongated tangentially. Again, you see the cytological characteristics I described: The dark cells have their maximum stain uptake in the cytoplasm near the center of the egg. At even earlier stages (figure a), the results are not quite clear. These cytological observations don't provide evidence for the existence of two categories of differentiated blastomeres before the 8 cell stage is reached.



Mulnard: That's the trouble with this problem – there's very often a conflict between morphological and physiological experimental data. I think the solution will be to put morphologists and physiologists together, and have them work on the same subject.

Dalcq: I just wish to express an opinion. The fotos that Mr. *Denker* has shown you are a new argument in favor of the existence of some pre-differentiation in the cytoplasm of the mammalian egg. And so, as has been said and repeated by Prof. *Mulnard,* we have this difficulty, this duality of positions, that there are the data relative to normal development without any intervention, observational data; and then there are the experimental data which seem and are largely opposed to the first group. I wish just to remind you that this opposition is not limited to mammalian eggs. It exists in amphibians, it exists in echinoderms, it exists in the several groups of eggs where *normal* development and regulation could be studied. So it seems to me that we have two expressions of one truth which we cannot distinguish, one from another. Now it appears from Dr. *Mulnard's* film that here, as in other cinematographic documents, the material is submitted to an agitation which implies considerable transformation. On the other hand, it is largely known that, in several groups, especially echinoderms and amphibians, there is a pre-elaboration of the key-macromolecules, the messenger RNA and so on, which are ruling the first stages of development. We should advise to study more eagerly what the factors of normal evolution are, not only those located in the nucleus, but also those of the cytoplasm with its various organelles and the cell membrane. The same processes should then be analysed step by step in the experimental situations where regulation takes place. And I dare insist that, in my opinion, what happens in the egg membrane and in the membranes of the blastomeres might be quite important.

Fig. 1. Differentiation of two groups of blastomeres during cleavage. Rabbit eggs, fixed by formol – alcohol – acetic acid, paraffin sections, stained by mercury-bromphenol blue (200 x).
a) 6-cell stage, 35 h p.c. One blastomere (at the top) is only lightly coloured, the other two react stronger with maximum stain uptake in the center of the egg.
b) 8-cell stage, 35 h p.c. The formation of a cap of flattened dark cells begins.
c) 40 h p.c.
d) and e) 63 h p.c. The dark cells surround the group of light ones to an extent varying from one egg to the other.
f) 3 d p.c. Egg from the Fallopian tube.
g) 3 d 4 h p.c. Egg from the uterus. A split begins to form between the two groups of blastomeres.
h) 3 d 8 h p.c. The split has enlarged to the blastocyst cavity. The embryonic knot cells are lightly coloured, the trophoblast cells dark.
(From *Denker, H.W.*, 1969: Dissertation Nat. Fak. Marburg, Zool. Jb. Physiol. *75*, 246–308 (1970), by courtesy of the editor.)

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